ABSTRACT

Title of Thesis: EFFECTS OF FULL-SCALE THERMAL HYDROLYSIS-ANAEROBIC DIGESTION ON THE TEMPORAL TRENDS OF POLYBROMINATED DIPHENYL ETHERS IN BIOSOLIDS AND THEIR PHYSICAL AND BIOLOGICAL DEGRADATION DURING WASTEWATER TREATMENT

Taylor Ann Motley, Masters of Science, 2020

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Biosolids produced at wastewater treatment plants (WWTPs) are rich in recovered nutrients and are often recycled through soil amendment on agricultural land. Advanced solids treatment strategies, including thermal hydrolysis pretreatment (THP) and anaerobic digestion (AnD), are utilized to produce cleaner, safer biosolids based on EPA classifications. Despite the phase-out of the flame retardant polybrominated diphenyl ethers (PBDEs) from commercial use in the U.S., they are

still present in biosolids and can be degraded to toxic byproducts during solids treatment. Their transformation during solids treatment is not well understood. This work shows that while phase-outs of PBDEs did not affect their concentrations in biosolids from the target WWTP, the implementation of THP-AnD treatment in 2014 led to increased PBDE degradation during solids treatment. This significantly lowered PBDE concentrations and shifted congener distribution to favor smaller, more toxic congeners in final biosolids compared to lime-stabilized biosolids historically produced at the target WWTP. Comparisons between the target WWTP and other AnD facilities without THP revealed that more efficient PBDE degradation occurred during THP-AnD treatment despite lower abundances of debrominating bacteria in digesters. Future work will examine if PBDE degradation during THP-AnD treatment is due to physical or biological processes.

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by

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Chapter 1: Introduction

1.1 Biosolids

In wastewater treatment plants (WWTPs), the solids waste stream is often treated for future use as biosolids. Cake biosolids are the nutrient-rich final effluent from WWTPs and have a consistency similar to soil, with a solids content between 11-40% (Lu et al., 2012). In 2017 it was estimated that annual biosolids production had doubled in the U.S. to 13.84 dry million tons of biosolids (Seiple et al., 2017) compared to estimates of production in 2004 (Breecher et al., 2007). The goal of municipal solids treatment is often to reduce the volume of waste produced, recover nutrients from sludge, and produce a final effluent product that is reusable (Yaashikaa et al., 2020). Production of biosolids is conventionally a way to accomplish these goals. After being produced, biosolids can go to landfills, be incinerated, or applied to agricultural land as soil amendment (Roy et al., 2011). Incineration of biosolids can lead to emission of greenhouse gases, while disposal in landfill can lead to the leaching of hazardous wastewater effluent byproducts into the soil and groundwater (Joo et al., 2015).

Most recent national biosolids survey estimated that about 55% of biosolids produced are applied to agricultural land as soil amendment (Breecher et al., 2007). Their use as a fertilizer amendment can be beneficial for the environment in several ways and helps accomplish the wastewater treatment goal of reusing biosolids rather than disposing of the effluent. Biosolids contain nutrients and elements essential to

plant growth, including nitrogen, phosphorus, and a high organic carbon content (Alvarenga et al. 2015). Biosolids amendment has been shown to improve soil quality and crop yield (Alvarez-Campos and Evanylo, 2019; Fuentes et al., 2017). It also helps reduce greenhouse gas emissions from soil microbes, since the treated material in biosolids serves as a readily available carbon source that is much easier to digest than complex organic matter (Torri et al., 2014).

Environmentally safe reuse of biosolids through land application can be challenging due to problems posed by its source, wastewater. Wastewater influent streams are often a combination of human fecal matter, wash water, storm drain runoff, and industrial drainage. These sources can contaminate wastewater with harmful pathogens, heavy metals, nutrient pollutants, and persistent organic pollutants (POPs) like solvents, pharmaceuticals, and more (Roy et al., 2011). Public perception is still an obstacle for the reuse of biosolids as soil amendment. Odor plays a part in this and may be managed through different treatment strategies (Joo et al., 2015). There are many different treatment options for solid waste, and each can produce a different quality biosolids product. Biosolids use is restricted based on the quality of the final product.

The Environmental Protection Agency (EPA) has created regulations for how biosolids product can be applied to land based on a classification system outlined in 40 CFR (Title 40, Code of Federal Regulations) Part 503 Biosolids Rule (Part 503 Rule). This system ranks the quality of biosolids based on pathogen count, vector attraction, and concentration of certain metals. Class A biosolids have the strictest regulations and are therefore considered safest for the environment and ecosystem.

Class A regulations mandate that biosolids meet the following standards: i) either a density of Salmonella sp. less than 3 MPN (most probable number)/4 grams dry weight or a fecal coliform density less than 1000 MPN/g dry weight (d.w.), ii) vector attraction reduction through entire virus density below 1 plaque-forming unit per 4 g dry weight, and iii) the concentrations of metal pollutants, including arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc, must be below a ceiling concentration limit put forth in the EPA's federal rule (U.S. EPA, 1994). WWTPs can also produce exceptional quality (EQ) biosolids when the final product exceeds the regulations set forth for Class A ranking. Class B biosolids can contain a higher level of pathogens at up to 2 million MPN/g d.w. of biosolids (Lu et al., 2012). EQ Class A biosolids can be applied to land used for growing food crops and is ruled safe for human contact by the EPA. It can also be used in home gardens and public contact areas. Class B use is more restricted and is generally used on farmland growing animal feed (Lu et al., 2012).

1.2 Lime stabilization and thermal hydrolysis pretreatment (THP) to anaerobic digestion (AnD)

The WWTP at the center of this research is located in the Mid-Atlantic region of the U.S. It is the largest advanced wastewater treatment in the world and has a treatment capacity of 1.4 million m^3/day (Wang, 2017). Wastewater received at this facility is mostly from residential sources, but influent from combined sewer/stormwater drainage systems is received as well (Andrade et al., 2015). Two final effluent streams are produced from this facility, clean water discharged into a nearby river and nutrient-rich biosolids. Historically, the target WWTP has produced

a Class B biosolids product stabilized by the addition of lime. Lime, or $CaCO_3$, is a chemical method of sludge treatment. This method meets pathogen level restrictions outlined in EPA Part 503 Rule by raising the pH of biosolids to above 12 (Fytili and Zabaniotou, 2008). The target WWTP typically added lime on a 15% dry weight basis. In November of 2014, the target facility implemented a new stabilization treatment process that includes the Cambi™ Thermal Hydrolysis Pretreatment (THP) and anaerobic digestion (AnD). The new system was adopted with the intent to reduce the volume of solids produced during treatment and increase biogas production during AnD through enhanced microbial activity. Advancements in solids treatment were made in order to decrease pathogen levels in biosolids and produce an EQ Class A biosolids product.

Anaerobic digestion can be implemented in municipal waste treatment to accomplish these treatment goals. These reactors support a complex microbial community. During AnD, influent sludge enters the oxygen-free reactors and is continuously stirred at a temperature of about 37°C for a retention time of 2-3 weeks (Halsey III, 2014). The influent stream is comprised mainly of large organic polymers like polysaccharides, lipids, and proteins. A diverse community of microbes work together to break down these molecules into monomers through hydrolysis reactions. The resulting products are simple sugars, amino acids, and fatty acids. Acidogenic bacteria further break down monomers into volatile fatty acids (VFAs) and simple alcohols, with H_2S, CO_2, and NH_3 as byproducts. VFAs and alcohols are transformed to H_2, CO_2, and acetic acid through acetogenesis. Finally, acetic acid is

fully oxidized into CO_2, H_2O, and CH_4 by archaea in a process called methanogenesis (Gavala et al., 2003).

This treatment strategy helps the target WWTP produce EPA-approved biosolids. Volatile organic matter is reduced during anaerobic digestion, which helps remove the source of energy for pathogenic organisms and promote the growth of non-pathogens. Pathogenic bacteria get outcompeted in this way, effectively lowering their density in the final biosolids product. High temperatures in the digester also work to reduce pathogens, which in turn lowers vector attraction (U.S. EPA, 2003). WWTPs can gain financial outcomes from treating sludge in this manner and meeting EPA requirements. AnD helps produce sellable biosolids product that is available to a wider range of customers. Volume of sludge is also reduced during microbial digestion, allowing WWTPs to save valuable space by needing to store less effluent. An important benefit of AnD to WWTPs is the savings on energy cost that can come from using AnDs for sludge treatment. The final step in digestion is the production of methane gas by archaea, and this gas can be captured and turned into electricity. The target WWTP is able to produce 10-15 MW of electricity a year in this manner, and it powers a third of their entire plant (Halsey III, 2014). Since this facility is the largest WWTP of its type in the world, this translates to a significant amount of money saved on electricity.

Pretreatment technologies used in connection with AnD can help WWTPs more efficiently meet their treatment objectives, which often include i) removing harmful bacteria, ii) reducing the volume of sludge, and iii) saving money through biogas production. The target WWTP uses thermal hydrolysis pretreatment (THP)

prior to AnD to accomplish these goals. THP treatment involves sludge flowing into a series of reactors where it is flashed with high heat (about 180°C) and high pressure (55-138 psi), followed by a pressure drop in the final step. This process helps solubilize organic matter in the sludge influent stream by breaking down large organic polymers into simpler molecules. Cell walls of microbes are burst during this process, helping to reduce the density of harmful bacteria in the effluent (Bougrier et al., 2008). THP pretreatment can reduce the overall volume of sludge by releasing water as steam during the process. The steam can be recycled to heat the reactors so that the system requires no outside energy.

Anaerobic digestion is made more efficient with THP as a pretreatment, since it changes the biochemistry occurring in the digesters. Hydrolysis, the first step to occur during anaerobic digestion of solids, is considered to be the rate-limiting step of the digestion process (Kallistova et al., 2014). With THP pretreatment, polymers are solubilized into monomers before entering the digesters. Monomers serve as a much more bioavailable source of energy for the digester's microbial community, allowing full digestion to occur much more quickly. With the process more efficient, production of methane gas increases, more electricity can be generated, and retention time of sludge can be lowered.

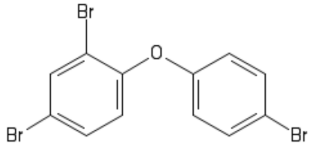
1.3 Polybrominated diphenyl ethers (PBDEs)

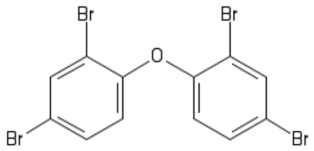
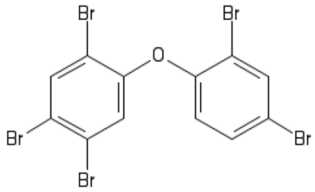
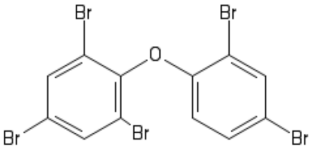
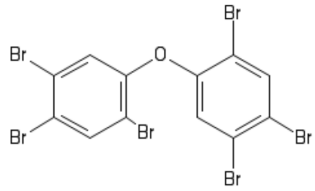
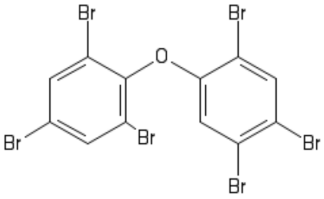
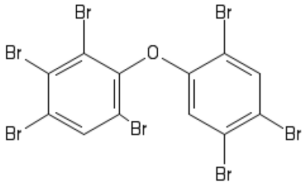
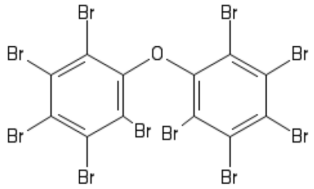
Polybrominated diphenyl ethers (PBDEs) are a class of industrially-produced aromatic organic compounds with a diphenyl ether skeleton that can be brominated in up to 209 different configurations (Fromme et al., 2009). Each configuration is referred to as a PBDE congener. PBDEs were used globally as flame retardants in

many different commercial products since their introduction to the market in the 1970s. Three main commercial formulations of PBDE congeners were historically used. Penta-BDE formulations were mainly used in the production of furniture, octa-BDE mixes were used in plastics, and deca-BDE was used to make electronics (Daso et al., 2010). Deca-BDE formulations were estimated to be responsible for 83.3% of global demand for PBDEs in 2001 (La Guardia et al., 2006).

The contribution of each PBDE congener to commercial formulations was determined by La Guardia et al. in their 2006 study. Penta-BDE is primarily comprised of BDE-47 and BDE-99, as well as BDE-100, BDE-153, and BDE-154. Octa-BDE formulations vary, but mainly consist of BDE-183, BDE-209, and other octa-BDEs, depending on the product. Deca-BDEs are made almost entirely of the fully brominated congener BDE-209. The structure of these congeners are shown in Table1. Due to their toxicity and wide environmental presence, manufacturers and importers began to voluntarily phase PBDEs out of production. By 2006, octa- and penta-BDE had been phased out of use in the US, while the phase-out of deca-BDE, the most widely used formula, began in 2009 and was not complete until 2013.

Table 1. Structures and properties of eight PBDE congeners.

Congener	Structure	Molecular Weight (g/mol)	Log K_{ow}[a]
BDE-28		406.89	5.96

BDE-47		485.79	6.66
BDE-99		564.7	7.12
BDE-100		564.7	7.31
BDE-153		643.6	7.48
BDE-154		643.6	7.70
BDE-183		722.5	8.35
BDE-209		959.2	9.16

[a] Data obtained from Yue and Li, 2013.

PBDEs have been shown to pose health risks to humans, especially infants and small children. Studies have linked toxic levels of PBDEs in women's bloodstream to lower fertility rates and found that PBDEs tend to accumulate in

breastmilk (Herbstman et al., 2010). Prenatal & childhood exposure to PBDEs leads to significant detrimental impacts on child neurobehavioral development (Eskanazi et al., 2013). Although these chemicals are no longer being produced or imported, they are still of great concern due to their widespread presence in products and materials found in homes and buildings throughout the US (Sjodin et al., 2008; Fromme et al., 2009). PBDEs are often added into the last steps of manufacturing and can leach off products readily and partition to dust particles or skin. From there, they are free to enter the WWTP inlet stream through sewers and storm drains.

PBDEs are hydrophobic molecules with log K_{ow} of approximately 6 to 10, depending on the congener (Table 1). They therefore partition to solid organic matter upon entering wastewater treatment facilities and are found mostly on the solids treatment side of wastewater treatment streams. PBDEs are routinely found in biosolids in North America (Song et al., 2006; Kim et al., 2013; Davis et al., 2015; Andrade et al., 2015), Australia (Gallen et al., 2016; McGrath et al., 2020), Asia (Lee and Kim, 2017; Wu et al., 2017), and Europe (Zennegg et al., 2013; Knoth et al., 2007). EPA rules do not have regulations on the concentration of PBDEs in biosolids. It has been shown that the application of contaminated biosolids to agricultural land correlates to an accumulation of PBDEs in soil (Andrade et al., 2010), which can persist for years with little degradation (Sellstrom et al. 2005, Venkatesan and Halden 2014).

For this reason, biosolids are considered an important source of PBDEs to environmental media and ecosystem. They have been shown to accumulate in sediments (Da et al., 2019), earthworms (Sellstrom et al. 2005), small aquatic life (La

Guardia et al., 2007; Gandhi et al., 2017), and larger marine animals (Rotander et al., 2012). Smaller congeners have lower K_{ow} (Table 1) and adsorb to organic matter more weakly than higher brominated compounds. Their increased mobility in environmental media also increases the congeners' bioavailability to organisms. Smaller PBDE congeners are considered more toxic than parent molecules due to their greater tendency to bioaccumulate (Gandhi et al., 2011). It is therefore important to understand how PBDEs transform during municipal solids waste treatment.

Organohalide-respiring bacteria (OHRB) capable of biologically degrading POPs via reductive dehalogenation have been identified in AnD microbial communities (Zhao et al., 2020). Nelson et al. (2011) found that OHRB account for a very small percent of bacteria in digesters. OHRB are capable of transforming larger PBDEs to smaller congeners under anaerobic conditions (Huang et al., 2019; Lee et al., 2011; Robrock et al., 2008). During reductive debromination in AnDs, OHRB use H_2 as an electron donor, break the carbon-bromine bond on PBDE molecules, and replace the bromine with an H atom. Organic C found in small molecules are normally used as a carbon source for these bacteria (Lee et al., 2011). OHRB can compete for some of the same key substrates that methanogenic archaea use to produce methane gas. Like methanogens, debrominating bacteria also use H_2 as an electron source. While they can use other small organic molecules for carbon sources, studies have shown that complete dehalogenation occurs when acetic acid is used for carbon (Lee et al., 2011). This directly competes with one of archaea's main electron sources, although they too can use other small organic molecules like formic acid and methanol (Thauer, 1998).

Two OHRB bacteria known to degrade PBDEs (Huang et al., 2019; Lee et al., 2011; Robrock et al., 2008) are analyzed in this research and are known to be present in AnDs (Smith et al. 2015) and capable of degrading PBDEs (Lee et al., 2011; Robrock et al., 2008; Huang et al., 2019). They include the obligate phylogenies *Dehalobacter* and *Dehalogenimonas*, which exclusively utilize halogenated compounds for electron acceptors during respiration. Tokarz et al. (2008) proposed degradation pathways for major PBDE congeners based on their study of BDE-209 degradation byproducts in anaerobic sediments and biomimetic systems. This demonstrates how larger PBDE congeners can be transforms to smaller PBDEs during both physical and biological degradation.

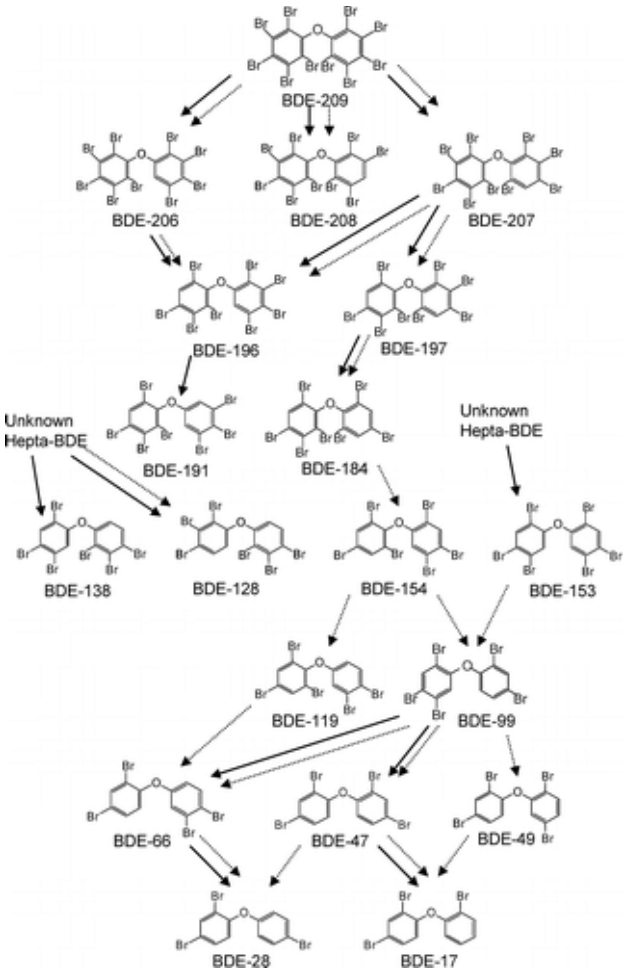


Figure 1. Proposed debromination pathway for PBDE congeners in anaerobic sediments (solid arrows) and biomimetic microcosms (dashed arrows) (Tokarz et al., 2008).

At the target WWTP, PBDEs may also be physically debrominated during THP pretreatment. However, there is little consensus on the effects of THP-AnD treatment on the degradation of persistent organic pollutants (POPs). Diaz et al. (2020) determined that lab-scale THP-AnD systems more effectively removed POPs than AnDs without pretreatment, but biodegradation of pollutants in AnDs alone was generally the same. Another study of pilot-scale THP-AnDs also concluded pretreatment did not substantially increase the biotransformation of POPs during digestion (Taboada-Santos et al., 2019). A study of POPs during full-scale THP-AnD treatment yielded mixed results, where removal of the antimicrobial triclocarban occurred during THP pretreatment (Armstrong et al., 2017a) but did not occur for phthalate plasticizers (Armstrong et al., 2017b). Removal of POPs from both full-scale studies were not efficient enough during anaerobic digestion to counterbalance the concentrating effect of solids reduction in AnDs (Armstrong et al., 2017a; Armstrong et al., 2017b).

1.4 Study Objectives

The work presented here focuses on the presence and transformation of PBDEs in WWTP sludge and biosolids. While few studies have shown how their levels in biosolids may have changed since being phased out of use (Hale et al., 2012; Andrade et al., 2015), it is still unknown how advanced solids treatments like THP-AD process may compound effects on long-term trends in their concentrations. In Chapter 2, the concentration of eight PBDE congeners were analyzed in both Class A and Class B biosolids over a 4-year period at the target WWTP. Trends of congener

concentrations over time are evaluated in order to assess the impact phase-outs of PBDEs may have had on their presence in biosolids. In addition, our study encompassed the advancement in solids treatment adopted by the target WWTP in 2014 and assesses its influence on the concentration and congener distribution of PBDEs in biosolids.

Chapter 3 assesses the possible physical and biological degradation of PBDEs occurring during full-scale THP-AnD treatment. No known studies have focused on the transformation of PBDEs and related OHRB abundances across full-scale THP-AnD systems. This study addresses the gap in knowledge by analyzing the concentrations of eight PBDE congeners in sludge samples collected at each step of a full-scale THP-AnD treatment train. The solids treatment train of two other full-scale AnD facilities were also sampled to assess if THP pretreatment coupled anaerobic digestion leads to more efficient degradation of PBDEs during solids treatment. A facility with aerobic digestion of sludge was also characterized. This study analyzes OHRB in each step of the four WWTPs with the goal of examining if THP pretreatment to AnD enhances the presence and abundance of dehalogenating bacteria compared to treatment systems without THP.

Chapter 2: Influence of thermal hydrolysis-anaerobic digestion on temporal trends of polybrominated diphenyl ethers in biosolids

Abstract

While biosolids are rich in recovered nutrients, they also contain persistent organic pollutants (POPs) that can leach into the environment when biosolids are land-applied. The flame retardant polybrominated diphenyl ethers (PBDEs) are consistently detected in biosolids and it is unclear how their phase-out from production in the early 2000s affects their long-term concentrations. Advanced solids treatment processes can also compound this problem since larger PBDE congeners can degrade to more mobile and toxic smaller congeners during treatment. There is a need to know how different policies and practices affect the concentration and degradation products of PBDEs in biosolids. Biosolids were sampled from a Mid-Atlantic wastewater treatment plant (WWTP) over a 4-year period and analyzed for polybrominated diphenyl ethers (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209). This study showed that the implementation of THP-AnD treatment system in 2014 at the target WWTP produced biosolids with less total amount of PBDEs, but with a higher fraction of smaller, more toxic congeners than found in lime-stabilized biosolids previously produced at this WWTP. Congener distributions indicated that increased degradation of PBDEs occurred during THP-AnD treatment compared to lime stabilization, as the distribution shifted to favor a

majority of lesser-brominated PBDEs in THP-AnD treated biosolids compared to distributions in lime-stabilized biosolids that favored BDE-209. Results also indicated that BDE-209 concentrations in biosolids produced at the target WWTP were unaffected by the phase-out of deca-BDEs from commercial markets, while the concentrations of penta-BDEs appear to have stabilized after previously decreasing following their phase-out in the early 2000s.

2.1 Introduction

Biosolids are the nutrient-rich product of treated wastewater effluent. Their nitrogen, phosphorus, and high organic carbon content (Alvarenga et al. 2015) can improve soil quality and crop production when land applied (Alvarez-Campos and Evanylo, 2019; Fuentes et al., 2017). In 2017 it was estimated that biosolids production has doubled in the U.S. to 13.84 dry million tons of biosolids (Seiple et al., 2017) compared to production in 2004 (Breecher et al., 2007). Biosolids are known to contain pollutants such as flame retardants, antimicrobials, pesticides, plasticizers, and pharmaceuticals (Andrade et al., 2015; Armstrong et al., 2017a; Clarke et al., 2010; Xia et al., 2005). Many of these pollutants are highly hydrophobic and partition to solid organic matter. Because 55% of biosolids produced are land-applied (Breecher et al., 2007) they can act as a source of contaminants to the environment.

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants used worldwide in a number of industries since the 1970s. Three commercial formulations consisting of a mixture of PBDE congeners were used historically. They include deca-BDE (mainly BDE-209), octa-BDE (mainly BDE-183, BDE-209, and other

octa-BDEs, depending on the product), and penta-BDE (mainly BDE-47 and BDE-99, as well as BDE-100, BDE-153, and BDE-154) (La Guardia et al., 2006). PBDE exposure has been linked to decreased fertility in women (Harley et al. 2010) and significantly impaired neurobehavioral development in children (Eskanazi et al. 2013). Due to their toxicity and wide environmental presence, manufacturers and importers began to voluntarily phase PBDEs out of production. By 2006, octa- and penta-BDE had been phased out of use in the US, while the phase-out of deca-BDE, the most widely used formula, began in 2009 and was not complete until 2013.

Despite their phase-out, PBDEs are still of great concern due to their widespread presence in the environment. They have been shown to accumulate in sediments (Da et al., 2019), household dust (Sjodin et al., 2008; Fromme et al., 2009), fish (Gandhi et al., 2017), and humans (Fromme et al., 2009; Harley et al. 2010; Eskanazi et al., 2013). PBDEs are also routinely found in biosolids in North America (Song et al., 2006; Kim et al., 2013; Davis et al., 2012; Andrade et al., 2015), Australia (Gallen et al., 2016; McGrath et al., 2020), Asia (Lee and Kim, 2016; Wu et al., 2017), and Europe (Zennegg et al., 2013; Knoth et al., 2007). Once biosolids are land-applied, PBDE can persist in soil for decades with little degradation (Sellström et al. 2005; Venkatesan and Halden, 2014).

In 2014, a Mid-Atlantic wastewater treatment plant (WWTP) at the center of this study implemented a new stabilization treatment process that includes the Cambi™ Thermal Hydrolysis Pretreatment (THP) and anaerobic digestion (AnD). The new system was adopted with the intent to reduce the volume of solids produced during treatment and increase biogas production during AnD through enhanced

microbial activity. Advancements in solids treatment were made in order to decrease pathogen levels in biosolids and produce a higher-quality Class A biosolids product based on the classification set forth by the U.S. Environmental Protection Agency (U.S. EPA, 1994). Class A biosolids can be applied to home gardens and public contact areas and have a much lower pathogen level and vector attraction than Class B biosolids (U.S. EPA, 1994). The THP-AnD process produces Class A biosolids and replaced the practice of lime stabilization used previously at the target WWTP to produce Class B biosolids. No regulations exist regarding the presence of PBDEs in biosolids. Several studies have shown that biological debromination transforms highly-brominated PBDEs to lower-brominated congeners under anaerobic conditions (Huang et al., 2014; Stiborova et al., 2015; Tokarz et al., 2008). While no studies have focused on the degradation of PBDEs specifically during thermal hydrolysis pretreatment, existing studies have shown that other POPs can be transformed during this treatment (Armstrong et al., 2017b; Diaz et al., 2020). Therefore, the THP-AnD treatment process could influence the concentration and distribution of PBDE congeners in biosolids. Since lower brominated PBDEs are considered to be more mobile and more toxic than higher brominated PBDEs (Liu et al., 2016), there is a need to know how different policies and practices affect the concentration and degradation of PBDEs in biosolids. While few studies have shown how their levels in biosolids may have changed since being phased out of use (Hale et al., 2012; Andrade et al., 2015), it is still unknown how advanced solids treatments like THP-AD process may compound effects on long-term trends in their concentrations.

A previous study by Andrade et al. (2015) evaluated long-term trends of PBDEs in Class B biosolids produced at this study's target WWTP from 2005 to 2011. Our study builds upon this work by analyzing the concentration of eight PBDE congeners in both Class A and Class B biosolids in an additional 4-year period. Trends over time are evaluated in order to assess the impact phase-outs of PBDEs may have had their presence in biosolids. In addition, our study encompassed the advancement in solids treatment adopted by the target WWTP in 2014 and we analyzed its influence on the concentration and congener distribution of PBDEs in biosolids.

2.2 Materials and methods

2.2.1 Target Compounds

Eight PBDE congeners were chosen for this study because of their widespread use in commercial formulas and their known presence in biosolids and the environment. They include BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209.

2.2.2 Sample Collection

The target WWTP is a large, Mid-Atlantic plant that historically used the addition of lime to stabilize their Class B biosolids product. Details of this facility's treatment process have been described previously (Armstrong et al., 2016). Grab samples of biosolids were collected following lime stabilization approximately every four months from February 2012 to November 2014. In November 2014, the target WWTP adopted a new solids stabilization strategy that used THP pretreatment and

AnD to produce Class A biosolids. Class A biosolids included in this study were collected by Wang (2017) each week from the final cake belt conveyor from November 2014 to February 2015 (start-up phase) and monthly from February 2015 until January 2016. All samples were collected in 250-mL solvent-rinsed amber glass jars with PTFE-lined plastic lids except for three of the Class B samples, which were collected in high-density polyethylene (HDPE) 250-mL jars. Samples were frozen at -40℃ until analysis.

2.2.3 PBDE Analysis

The total solids content of each sample was determined using EPA Method 1614 (U.S.EPA, 2010). Samples were dried for at least 12 hours in a 103-105℃ oven (Fischer Scientific, Isotemp, USA) and cooled in a desiccator.

PBDE analysis was based on EPA Method 1614 and a modified method adapted from Deng et al., 2015, Krol et al., 2012, and Giergielewicz-Mozajska et al., 2001. Samples were thawed overnight in a -4℃ refrigerator and brought to room temperature before beginning analysis. In preparation for extraction, samples were mixed with hydromatrix (Agilent Technologies, Hydromatrix, USA) using a mortar and pestle until a dry sample was produced. Each sample was spiked with 40 ng of the surrogate, PCB-209 (AccuStandard, PCB-209-CS, 99%, New Haven, CT, USA). Samples were extracted using an Accelerated Solvent Extraction (ASE) System 200 (Thermo Scientific, Dionex ASE 350 with Dionium Components Smartrun System & Solvent Saver System, Sunnyvale, CA). Two extraction cycles were performed at 120℃ and 2000 psi, using a solvent stream of 20% acetone (Fisher Scientific, Acetone, USA) and 80% hexane (Fisher Scientific, n-Hexane, USA). Each 10-minute

extraction was run at a constant temperature and pressure and produced about 35 mL of extract.

Extracts from the ASE were further cleaned to remove lipids and sulfur, which have been shown to interfere with PBDE analysis (Berton et al., 2016). Glass columns (Kimble™ Kontes™ PTFE-Plugged Column, 300-mm L x 22-mm ID, USA) were packed from bottom to top with glass wool (Acros Organics, Glass wool, New Jersey), 4 g copper powder (Fisher, Copper C434-500 Powder, USA), 1 g activated silica gel (Alfa Aesar, 150 angstroms wide pore silica gel, USA), 2 g 33% basic silica gel with NaOH (Fisher, NaOH pellets, USA), 1 g activated silica gel, 4 g 40% acidic silica gel with concentrated sulfuric acid (Fisher, A300-212 Sulfuric Acid, USA), 2 g activated silica gel, and 4 g anhydrous sodium sulfate (Fisher, S429-212 Sodium Sulfate Anhydrous, USA). Each sample extract was passed through a column with gentle air flow and hexane. The eluted cleaned extract was dried completely using a Zymark TurboVap LV Evaporator (Hopkinton, MA) under gentle N_2 flow, and then reconstituted in 1 mL of hexane and spiked with 40 ng of the internal standard, PCB-138 (Cambridge Isotope Laboratories, Inc., $^{13}C_{12}$ PCB-138-CS, 99%, Andover, MA, USA).

All samples were analyzed using an Agilent 6890N gas chromatograph (GC) coupled with an Agilent 5975 series mass selective detector (MSD) in negative chemical ionization mode (Agilent Technologies, USA). A constant flow of 1.6 mL/min of helium gas was delivered to an Agilent capillary column (DB-5MS) with a nominal length of 15.0 m, nominal diameter of 250 um, and nominal film thickness of 0.10 um (J&W Scientific, Folsom, CA). The oven temperature began at 48℃ and

ramped up to 310℃ for a run time of 22 minutes. A programmable temperature vaporizing inlet (GERSTEL, Inc., Linthicum, MD, USA) was used in pulsed splitless mode at an initial temperature of 51℃, and ramped up to 300℃ at a rate of 600℃/s. MSD source and quad temperatures were 150℃ each. A mix of the PBDE congeners of interest, including BDE-28 (97.9%), BDE-47 (98%), BDE-99 (99.1%), BDE-100 (100%), BDE-153 (100%), BDE-154 (100%), BDE-183 (98.8%), and BDE-209 (98.3%), was obtained from AccuStandard (New Haven, CT, USA). Target compounds were quantified using selective ion monitoring and a 9-point calibration curve.

2.2.4 Quality control

Method detection limits (MDLs) and quantification limits (QLs) were calculated according to Carden, 1988 and determined by Wang (2017) (Table 2). QLs are three times MDLs. All samples had concentrations of BDE-47, BDE-99, BDE-100, and BDE-209 above QL. BDE-28 was excluded from analysis as 77% (n=30) of all samples in this study had concentrations below its MDL. All 9 Class B biosolids samples had BDE-183 concentrations below MDLs, while concentrations in Class A samples (n=21) were all above QLs. Concentrations of BDE-153 were above QLs in 97% of samples, while 93% of samples had concentrations of BDE-154 above QLs.

Table 2. Method detection limits (MDL) and quantification limits (QL) of surrogate standard (PCB-209) and each PBDE congener.

	PCB-209	***BDE-28***	***BDE-47***	***BDE-99***	***BDE-100***	***BDE-13***	***BDE-154***	***BDE-183***	***BDE-209***
MDL (ug/kg d.w.)	6.026	1.431	1.172	1.006	1.087	0.911	0.666	0.996	5.731
QL (ug/kg d.w.)	18.077	4.292	3.515	3.017	3.261	2.733	1.998	2.988	17.192

PCB-209 recoveries in Class A samples were high and steady, averaging 103.0±5.0% (n=53). However, PCB-209 recoveries in Class B samples began at 111.7% and decreased steadily to 33% in the order that samples were analyzed by GC-MSD (Appendix A). The pattern in decreasing surrogate recoveries suggested possible interference during GC-MS analysis, perhaps due to matrix residue building up in the GC column throughout the run. Experiments not shown here have indicated that rinsing the column with hexane between biosolids samples helped improve PCB-209 recoveries in biosolids samples. However, Class B samples could not be rerun using this technique as they were erroneously disposed of after their initial analysis. Concentrations were therefore corrected for surrogate recovery in all samples so that meaningful comparisons could be made between Class A and Class B samples.

Each batch of samples included a blank as well as sand and matrix sample spiked with 40 ng of surrogate and a BDE-Mix of approximately 7 ng BDE-28, 20 ng BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183, and 140 ng BDE-209 (Cambridge Isotope Laboratories, Inc., Andover, MA and (AccuStandard, New Haven, CT, USA). All blanks in this study showed no contamination. Average surrogate recovery was 67.00% in the sand spike and only 5.50% in the matrix spike of the Class B samples batch, while average PBDE recovery was 117.3±58.8% and

545.1±285.1% in sand and matrix spikes, respectively. Class A samples were processed in either 2 or 4 replicates, while Class B samples were run in one batch without replicates. Figures and statistical analysis were done using GraphPad Prism 6 software (GraphPad Software, Inc., San Diego, CA, USA).

2.3 Results and discussion

2.3.1 Overview of findings

This study analyzed 9 Class B biosolids samples collected from February 2012 to November 2014. In addition, data from 21 Class A biosolids samples collected between November 2014 to January 2016 from the target WWTP and analyzed by Wang in their 2017 study were also included in results presented here. The average total solids content was 36.06±3.64% (n=9) for Class B samples and 29.32±2.30% (n=21) for Class A samples. Concentrations of PBDEs in Class B biosolids were corrected to account for the differences in solids treatment between samples. THP-AD treatment of sludge leads to 65% reduction in mass in Class A biosolids compared to Class B. Additionally, lime was added to Class B biosolids on a dry weight basis of 15%. In total, concentrations of PBDEs were corrected for the 80% dilution of Class B biosolids compared to Class A.

Concentrations of PBDEs varied widely between Class A and Class B biosolids sampled in this study. Total PBDEs, including BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-209, averaged 3871±579 ug/kg d.w. (n=9) in Class B biosolids and 678.92±90.53 ug/kg d.w. (n=21) in Class A biosolids. In Class B samples, an average of 2656±577 ug/kg d.w. (n=9) BDE-209 was measured, while an

average of 277.0±52.7 ug/kg d.w. (n=21) BDE-209 was measured in Class A biosolids samples. Our findings generally fell within the same order of magnitude of BDE-209 concentrations found by other studies, especially for Class B biosolids. Concentrations in Class A tended to be lower. PBDE concentrations in biosolids have been shown to be highly variable geographically in the US. The Targeted National Sewage Sludge Survey (TNSSS) completed in 2009 is the largest and most recent representative sampling of biosolids in the US. BDE-209 was found at an average concentration of 2181±3463 ug/kg d.w. Biosolids sampled in 2010 in North Carolina (Davis et al., 2015) had an average BDE-209 concentration of 1823±127 ug/kg d.w, comparable to concentrations in this study's Class B samples. BDE-209 concentrations were 4-7 times higher in biosolids sampled in Chicago between 2004-2007, ranging from 4250 to 7840 ug/kg d.w. (Hale et al., 2012). Globally, BDE-209 concentrations in biosolids varied considerably as well. McGrath et al. (2020) reported a median concentration of 1800 ug/kg d.w. in biosolids collected from 15 different WWTPs in Western Australia from 2017 to 2018, but BDE-209 was found as high as 18,000 ug/kg d.w.

In this study, the average of the sum of BDE-47 and BDE-99 was 1034±184 ug/kg d.w. (n=9) in Class B biosolids and 369.1±53.7 ug/kg d.w. (n=21) in Class A samples. Levels found in Class B samples were detected at about the same order of magnitude as found in biosolids elsewhere in other North America, while concentrations in Class A biosolids were generally lower. Sludge surveyed in Ontario, Canada in 2006 had concentrations of BDE-47+BDE-99 between 947 and 2238 ug/kg d.w. (Song et al., 2006). In British Columbia, biosolids sampled between

2006 and 2007 were found to have 722 ug/kg d.w. of BDE-47+BDE-99 (Gorgy et al. 2013). TNSSS (2009) reported an average of 1426±748 ug/kg d.w. BDE-47+BDE-99 in U.S. biosolids.

2.3.2. Congener Distribution

PBDEs detected in this study consisted primarily of BDE-47, BDE-99, and BDE-209 (Figure 2). On average, these three congeners accounted for 95.22±1.15% (n=9) of PBDEs measured in Class B samples and 88.08±0.80% (n=21) of PBDEs in Class A samples. These three congeners have widely been reported to account for the majority of PBDEs measured in biosolids and sludge sampled from WWTPs in North America (Davis et al., 2015; Hale et al., 2012; Kim et al., 2013;), Germany (Knoth et al., 2007), and in Australia (Gallen et al., 2016; McGrath et al. 2020). Multiple t-tests of the congener's contribution percent confirmed that the distribution in Class A was significantly different than the distribution in Class B biosolids ($t(28.0)=22.20, 17.82, 15.43, 14.70, 12.68, 12.68$ in order of each congener, all p values < 0.0001). Class B samples consisted of 68.07±6.68% (n=9) BDE-209, indicating that deca-BDE was the most dominant congener contributing to PBDEs in biosolids from 2012 to 2014 at the target WWTP. In contrast, Class A biosolids contained 66.44±11.49% (n=21) of the congeners found in penta-BDE commercial formulas (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154) (La Guardia et al., 2006).

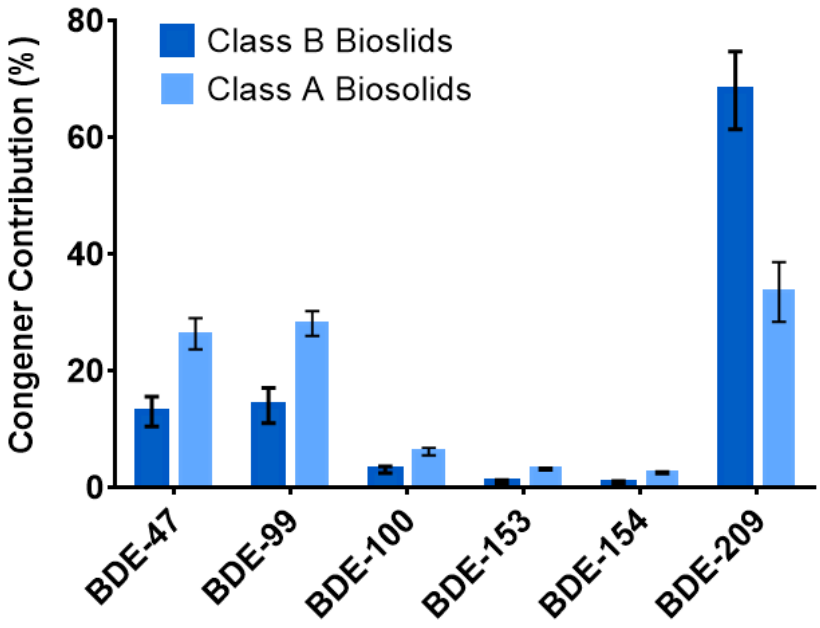


Figure 2. Average contribution of each congener to the total concentration of PBDEs in Class A and Class B biosolids collected from a Mid-Atlantic WWTP from 2012 to 2016.

2.3.3 Temporal Trends

The concentrations of PBDEs in each class of biosolids did not change significantly over time during the sampling periods. Although the phase-out of deca-BDE from production in the US, which began around 2009 and concluded in 2013, occurred during the Class B sampling period of this study, our results did not indicate a decrease in BDE-209 concentrations over time in final biosolids. The average concentration of BDE-209 in Class B biosolids was 2656±611 ug/kg d.w. (n=9) (Figure 3a). Concentrations of BDE-209 in biosolids are known to be variable (TNSSS, 2019; McGrath 2020), but studies investigating temporal trends of BDE-209

in U.S. biosolids during it's phase-out from 2009 to 2013 are limited. Andrade et al. (2015) sampled Class B biosolids from the same target WWTP between 2005-2011 and also found BDE-209 concentrations to vary but not change significantly over time. When corrected for solids content (as Class B concentrations presented here were corrected), the concentration of BDE-209 averaged 10,250 ± 3120 ug/kg d.w. in their 2015 study. The difference in BDE-209 concentrations between historical Class B biosolids (Andrade et al., 2015) and samples presented here are likely due to the different analytical methods used in each study rather than the phase-out of deca-BDE, as concentrations in each study showed no appreciable change over time during each multi-year sampling period.

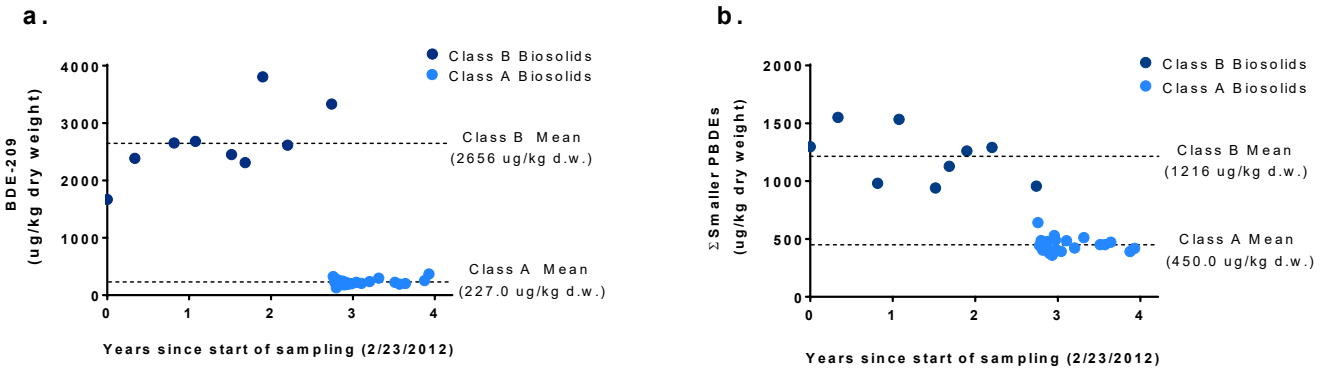


Figure 3. Average concentration of a) BDE-209 and b) smaller PBDEs in biosolids collected from a Mid-Atlantic WWTP between 2012 and 2016. Smaller PBDEs represent the sum concentration of BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154. Dotted lines represent the average PBDE concentration in each group of biosolids.

Studies investigating the temporal trends of PBDE congeners commonly found in penta-BDE commercial formulations have indicated that their concentrations

in U.S. biosolids samples were affected during their phase-out from 2004 to 2006. A study by Hale et al. (2012) suggested that concentrations of penta-BDEs decreased in biosolids collected from Chicago WWTPs between 2004-2007. Andrade et al. (2015) reported a 42% decrease in BDE-47+BDE-99 concentrations in Class B biosolids collected from this target WWTP over a 7-year period between 2005-2011. However, the concentration of the sum of smaller PBDE congeners (∑smaller PBDEs) measured in this study, including the congeners BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154, showed no significant change over time in Class B biosolids from 2012-2014 (Figure 3b). This indicates that penta-BDE concentrations likely stabilized in biosolids at the target WWTP following the penta-BDE phase-out from 2004-2006.

Concentrations of PBDEs measured in this study decreased significantly in biosolids following the target WWTP's implementation of the THP-AnD treatment system in 2014. Total PBDEs, including the sum of all congeners measured in this study, decreased significantly ($t(8.086)=15.62$, $p<0.0001$) by 82.46% after the change in treatment and remained steady after 2014. Average total PBDEs were 3871±614 ug/kg d.w. (n=9) in Class B biosolids but only 664.5±67.0 ug/kg d.w. (n=21) in Class A biosolids. All PBDE congeners measured here decreased following the change in treatment, but to different extents. BDE-209 concentration averaged 227.0±54.0 ug/kg d.w. (n=21) in Class A biosolids. This represents a statistically significant decrease ($t(8.05)=11.89$, $p<0.0001$) of 91.45% from the mean concentration of BDE-209 in Class B biosolids (Figure 3a). This indicates that BDE-209 may have degraded to smaller PBDE congeners during THP-AnD treatment, since the decrease in its concentrations occurs immediately following the change in solids treatment

technology at this WWTP. BDE-209 can be biologically degraded under anaerobic conditions (Gerecke et al., 2006; Tokarz et al., 2008; Huang et al., 2014) and has been shown to degrade in anaerobic digesters (Shin et al., 2010; Gerecke et al., 2006). Other persistent organic pollutants (POPs) have been shown to degrade during thermal hydrolysis pretreatment (Armstrong et al., 2017b; Diaz et al., 2020), and although PBDEs specifically have not yet been studied, the possibility of their degradation during this process should not be discounted.

Average ∑smaller PBDEs concentration was 1216±233 ug/kg d.w. (n=9) in Class B biosolids but only 450.0±63.7 ug/kg d.w. (n=21) in Class A biosolids (Figure 3b). This also represented a statistically significant decrease ($t(8.519)=9.718$, $p<0.0001$) of 63.24% from Class B to Class A biosolids. These congeners are also known to be degraded through biological reductive debromination (Huang et al., 2014; Robrock et al., 2008), and this process has been shown to occur in pilot-scale AnDs (Shin et al., 2010). Results show that the decrease in concentration was less in ∑smaller PBDEs (68.24%) than in BDE-209 (91.45%) (Figure 3), suggesting that BDE-209 may have debrominated at a faster rate than smaller PBDE congeners measured in this study. This is in agreement with other studies that have investigated the biological debromination of PBDE congeners in anaerobic environments, which concluded that the rates of PBDE degradation generally increase with increasingly brominated congeners (Tokarz et al., 2008; Shin et al., 2010; Huang et al., 2014). The 68.24% decrease in ∑smaller PBDEs in Class A biosolids versus Class B represents the degradation of those congeners during THP-AnD treatment, but the percent was also likely inflated by the formation of those congeners as larger PBDEs

debrominated during THP-AnD treatment. A proposed degradation pathway of biological PBDE debromination by Hunag et al. (2014) indicates that BDE-209 degrades to BDE-154, BDE-100, BDE-99, BDE-47 only with BDE-183 as an intermediate step. BDE-183 was found in concentrations below MDLs in Class B biosolids but at low concentrations in Class A biosolids, averaging 7.302±1.265 ug/kg d.w. (n=21). This indicates that larger PBDE congeners, including BDE-209 may be degrading to smaller congeners such as BDE-183 during THP-AnD treatment. Since BDE-183 is found at such low levels in Class A biosolids, this congener may not be the endpoint of BDE-209 degradation. It may be debrominated further to smaller PBDE congeners, including those measured in this study. ∑Smaller PBDEs may also have been formed as other nona-, octa-, and hepta-BDEs (Tokarz et al., 2008) already present in sludge but not measured in this study degraded during THP-AnD treatment.

To investigate how the PBDE congener distributions in biosolids were affected by the implementation of the THP-AnD treatment system, concentrations were normalized to account only for the diphenyl ether (DE) ring of each PBDE molecule (Equation 1).

$$Eq.\,1\text{: Congener concentration} \times \frac{\text{DE ring molecular weight (170.2 g/mol)}}{\text{PBDE congener molecular weight}}$$

Normalizing the values ensures that congeners' concentrations can be compared without their different molecular weights skewing the values. Changes in concentrations between the two classes of biosolids can therefore more accurately be assessed for the occurrence of PBDE degradation during the new treatment.

The ratio of ∑smaller PBDEs to BDE-209 was calculated for each sample using the normalized concentrations, shown in Figure 4. The ratio averaged 0.8708±0.2877 (n=9) in Class B biosolids, indicating that BDE-209 accounted for the majority of PBDEs in those samples. This ratio increased significantly ($t(27.00)=15.07$, $p<0.0001$) in Class A biosolids to 3.526±0.661 (n=20, one point removed based on results of Robust regression and outlier removal test, Q=1%), indicating that PBDE congener distributions in biosolids produced after the implementation of THP-AnD treatment shifted to favor smaller, less-brominated PBDEs. This likely reflects the increased degradation of PBDEs that occurred during the THP-AnD system compared to any degradation that may have occurred during lime stabilization at this WWTP. Debromination of PBDEs is not thought to occur due to the addition of lime. Instead, this process lowers the concentration of PBDEs in biosolids by diluting the solids, but congener distributions have been shown to remain unchanged (Kim et al., 2013).

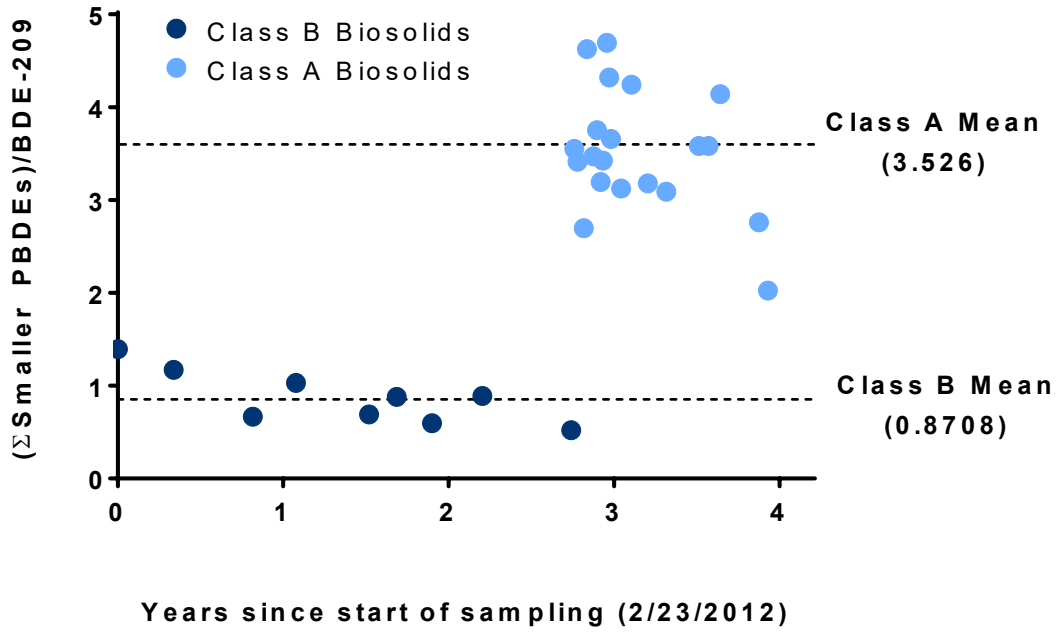


Figure 4. Average ratio of the sum of smaller PBDE congeners to BDE-209 measured in biosolids from a Mid-Atlantic WWTP between 2012-2016. Smaller PBDE congeners include BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154. Concentrations were first normalized to account only for the diphenyl ether ring of each congener. Dotted lines represent the average ratio of each class of biosolids.

Biosolids produced at the target WWTP are used as a soil amendment for agricultural lands. Results presented here show that the concentrations of all PBDE congeners decreased in THP-AnD-treated biosolids produced at the target WWTP compared to concentrations in lime-stabilized biosolids, initially indicating less PBDE contamination. However, THP-AnD-treated biosolids had a higher fraction of smaller, more toxic PBDE congeners compared to BDE-209 than found in lime-stabilized biosolids. PBDE congeners that are less brominated than BDE-209 have lower K_{ow} values (Yue and Li, 2013), bind to organic matter less preferentially, and

can therefore move into other environmental media more easily once contaminated biosolids are land-applied. Smaller PBDE congeners, including penta- and hexa-BDEs measured here, are known to be more toxic to plants than BDE-209 (Sun et al., 2020). Therefore, evaluating if THP-AnD-treated biosolids studied here are less harmful to the environment in terms of PBDE contamination compared to lime-stabilized biosolids cannot be adequately determined with only the results presented here.

2.4 Conclusions

Though there are many benefits to the application of nutrient-rich biosolids to agricultural land, their contamination by persistent organic pollutants like PBDEs raises a pressing environmental concern. The early 2000s phase-out of PBDEs from production in the U.S. aimed to cease the introduction of the toxic chemical to markets and in turn lower their presence in wastewater effluent. However, advanced solids treatment strategies can degrade PBDEs and lead to the production of smaller, more toxic PBDE congeners. This study showed that the implementation of THP-AnD treatment system in 2014 at the target WWTP produced biosolids with less total amount of PBDEs, but with a higher fraction of smaller, more toxic congeners than found in lime-stabilized biosolids previously produced at this WWTP. Congener distributions indicated that increased degradation of PBDEs occurred during THP-AnD treatment compared to lime stabilization, as the distribution shifted to favor a majority of lesser-brominated PBDEs in THP-AnD treated biosolids compared to distributions in lime-stabilized biosolids that favored BDE-209. Results also indicated that BDE-209 concentrations in biosolids produced at the target WWTP were

unaffected by the phase-out of deca-BDEs from commercial markets, while the concentrations of penta-BDEs appear to have stabilized after previously decreasing following their early 2000s phase-out. It is important to understand the transformation of POPs such as PBDEs that occurs during solid waste treatment. The degradation products can enter the environment and eventually our food chain when contaminated biosolids are land-applied. Future research will focus on the influence of individual steps of the THP-AnD process to assess the physical and biological debromination that occurs during solids treatment.

Chapter 3: Analysis of physical and biological degradation of PBDEs in full-scale wastewater treatment plants utilizing thermal hydrolysis pretreatment and anaerobic digestion

Abstract

It is important to understand how organic contaminants are transformed during full-scale municipal solids treatment. No known studies have focused on the transformation of polybrominated diphenyl ethers (PBDEs) and related organohalide-respiring bacteria (OHRB) abundances across full-scale thermal hydrolysis pretreatment (THP) to anaerobic digestion (AnD) systems. This study quantified eight PBDE congeners and abundances of *Dehalogenimonas* in the final biosolids product of one wastewater treatment plant (WWTP) with THP-AnD, two AnD WWTPs, and one aerobic digester (AeD) facility. Results of this study show that biosolids produced at the THP-AnD facility had a higher ratio of small PBDE congeners to BDE-209 (~3:1 ratio) than biosolids produced at other facilities in this study (~1:1 ratio). Penta-BDE congener ratios in THP-AnD final biosolids were most significantly different from ratios in commercial formulations as compared to other facilities' biosolids. While results seem to indicate more efficient debromination during THP-AnD treatment, AnDs at this facility did not have the highest abundances of the OHRB *Dehalogenimonas.* Results of this study do not suggest that THP pretreatment of sludge increases *Dehalogenimonas* abundance in anaerobic digesters. More efficient debromination occurring during THP-AnD solids treatment may be due to physical degradation during THP pretreatment rather than biological debromination during anaerobic digestion. In future work, congener ratios will be

compared in samples collected along the complete solids treatment train of these four facilities. Step-to-step comparisons of PBDE concentrations and OHRB abundances for each part of the solids treatment train will indicate at which point degradation is occurring, and may indicate whether it is due to physical or biological processes.

3.1 Introduction

Widespread use of brominated flame retardants in consumer goods has led to their presence in wastewater treatment plants' (WWTPs) sludge and biosolids (Song et al., 2006; Knoth et al., 2007). Polybrominated diphenyl ethers (PBDEs), a class of flame retardants, were phased out production in the early 2000s (USA EPA, 2006) due to their known toxic effects on humans (Harley et al. 2010; Eskanazi et al. 2013). Despite this, evidence of declining concentrations of PBDEs in WWTPs is inconclusive and they continue to be detected in WWTPs after their phase-out from production (Hale et al., 2012; Andrade et al., 2015).

Biosolids, the nutrient-rich final effluent from WWTPs, are used as soil amendment to improve soil quality and improve crop yields (Alvarez-Campos and Evanylo, 2019; Fuentes et al., 2016). It is estimated that 13.84 dry million tons of biosolids are produced each year (Seiple et al., 2017) and about 55% are land applied in the US (Breecher et al., 2007). PBDEs are hydrophobic molecules with log K_{ow} of approximately 6 to 10, depending on the congener (Yue and Li, 2013). They therefore partition to solid organic matter during wastewater treatment and are present in high concentrations in biosolids globally (Lee and Kim, 2016; McGrath et al., 2020; Knoth et al., 2007). Successive applications of contaminated biosolids to agricultural land

has correlated with an accumulation of PBDEs in soil (Andrade et al. 2010), which can persist for decades with little degradation (Sellstrom et al. 2005; Venkatesan and Halden, 2014).

Biological degradation of PBDEs can occur during wastewater treatment, as OHRB are known to be present in municipal anaerobic digesters (Smith et al., 2015). PBDEs can be biologically degraded by organohalide-respiring bacteria (OHRB) through reductive debromination in anaerobic environments (Huang et al., 2019; Lee et al., 2011; Robrock et al., 2008). No studies to date have examined degradation of PBDEs in sludge during THP pretreatment, but there is evidence that PBDEs can be thermally degraded in metal-catalyzed reactions. In their 2015 study, Li et al. showed that BDE-209 thermally degrades to smaller congeners (including BDE-183, BDE-154, BDE-100, and BDE-99) in metal-catalyzed reactions with Fe_2O_4 at 300°C. Degradation of larger PBDE congeners produces lower molecular weight molecules with varying degrees of bromination. Smaller congeners have lower K_{ow} (Yue and Li, 2013) and adsorb to organic matter more weakly than higher brominated compounds. Their increased mobility in environmental media also increases the congeners' bioavailability to organisms (Gandhi et al., 2011). Smaller PBDE congeners are considered more toxic than parent molecules due to their greater tendency to bioaccumulate (Gandhi et al., 2011). They have been shown to accumulate in sediments (Da et al., 2019), earthworms (Sellstrom et al. 2005), small aquatic life (La Guardia et al., 2007; Gandhi et al., 2017), larger mammals (Rotander et al., 2012), and humans (Fromme et al., 2009; Harley et al. 2010; Eskanazi et al. 2013).

The major objectives of advanced wastewater treatment are to reduce the volume of solid waste, efficiently recover nutrients, and produce a biosolids product that is reusable after treatment (Yaashikaa et al., 2020). Anaerobic digestion of solids is a common treatment strategy used to meet these goals, as biogas production by methanogenic archaea can be harnessed to generate electricity (Yaashikaa et al., 2020). Thermal hydrolysis pretreatment (THP) to anaerobic digestion is a viable option to more efficiently digest municipal waste solids. THP pretreatment uses high heat (about 180°C) and pressure (55-138 psi) to solubilize large organic polymers in sludge. This step is considered to be the rate-determining step of anaerobic digestion (Kallistova et al., 2014). In doing so, this pretreatment increases the activity of methanogenic bacteria in digesters and boosts biogas production (Xue et al., 2015). It is unknown if THP to AnD better supports the growth and activity of OHRB in AnDs. There is also little consensus on the effects of THP-AnD treatment on the degradation of persistent organic pollutants (POPs). Diaz et al. (2020) determined that lab-scale THP-AnD systems more effectively removed POPs than AnDs without pretreatment, but biodegradation of pollutants in AnDs alone was generally the same. Another study of pilot-scale THP-AnDs also concluded pretreatment did not substantially increase the biotransformation of POPs during digestion (Taboada-Santos et al., 2019). A study of POPs during full-scale THP-AnD treatment yielded mixed results, where removal of the antimicrobial triclocarban occurred during THP pretreatment (Armstrong et al., 2017b) but did not occur for phthalate plasticizers (Armstrong et al., 2017a). Removal of POPs from both full-scale studies was not efficient enough

during anaerobic digestion to counterbalance the concentrating effect of solids reduction in AnDs (Armstrong et al., 2017a; Armstrong et al., 2017b).

It is important to understand how organic contaminants are transformed during full-scale municipal solids treatment. No known studies have focused on the transformation of PBDEs and related OHRB abundances across full-scale THP-AnD systems. This study addresses the gap in the field by analyzing the concentrations of eight PBDE congeners in sludge samples collected at each step of a full-scale THP-AnD treatment train. The solids treatment train of two other full-scale AnD facilities were also sampled to assess if THP pretreatment coupled to anaerobic digestion leads to more efficient degradation of PBDEs during solids treatment. A facility with aerobic digestion of sludge was also characterized. This study analyzes OHRB in each step of the four WWTPs with the goal of examining if THP pretreatment to AnD enhances the presence and abundance of dehalogenating bacteria compared to treatment systems without THP.

3.2 Materials and Methods

3.2.1 Wastewater treatment plants and sample collection

Samples were collected from the solids treatment train of four Mid-Atlantic WWTPs in the summer of 2019 and spring of 2020 (Table 3).

Table 3. Characteristics of the WWTPs included in this study

WWTP ID	***Solids Treatment Process***	***Date Collected***
THP-AnF	(Gravity thickening/dissolved air flotation thickening)*[c] → THP*[c] → AnD*[c] → dewatering by belt press*[c]	8/8/19 (PBDEs), 3/6/2020 (qPCR)
AnF1	Gravity thickening* → Primary AnD* → Secondary AnD* → dewatering by rotary press*[c]	8/6/2019
AnF2	(Gravity thickening/fermentation)*[c] → AnD*[c] → dewatering by belt press*[c]/dewatering by centrifuge	7/26/2019
AeF	Influent* → AeD* → dewatering by drying bed*[c]	7/23/2019

*[c] composite sample collected

* single sample collected

Samples were collected in 250-mL solvent-rinsed amber glass jars with PTFE-lined plastic lids for PBDE analysis, and in Falcon 50-mL Conical Centrifuge Tubes for qPCR analysis. Sampled were stored in a -40°C freezer within 3 hours of sampling. The total solids content of each sample was determined using EPA Method 1684. Samples were dried for at least 12 hours in a 103-105℃ oven (Fischer Scientific, Isotemp, USA) and cooled in a desiccator.

3.2.2 PBDE Analysis

Eight PBDE congeners were chosen for this study because of their widespread use in commercial formulas and their known presence in biosolids and the environment. They include BDE-28. BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209.

3.2.2.1 Sample extraction and instrumental analysis

Methods of PBDE analysis were described in detail previously (Chapter 2, Section 2.2.4). Briefly, samples were spiked with 40 ng of the surrogate, PCB-209

(AccuStandard, PCB-209-CS, 99%, New Haven, CT, USA), and extracted using an Accelerated Solvent Extraction (ASE) System 200 (Thermo Scientific, Dionex ASE 350 with Dionium Components Smartrun System & Solvent Saver System, Sunnyvale, CA). Extracts from the ASE were further cleaned using manually packed columns with copper powder, activated silica gel, basic silica gel, activated silica gel, acidic silica gel, and sodium sulfate, in this order. The eluted cleaned extract was dried completely using a Zymark TurboVap LV Evaporator (Hopkinton, MA) under gentle N_2 flow, and then reconstituted in 1 mL of hexane and spiked with 40 ng of the internal standard, PCB-138 (Cambridge Isotope Laboratories, Inc., $^{13}C_{12}$ PCB-138-CS, 99%, Andover, MA, USA). All samples were analyzed using an Agilent 6890N gas chromatograph (GC) coupled with an Agilent 5975 series mass selective detector (MSD) in negative chemical ionization mode (Agilent Technologies, USA). A mix of the PBDE congeners of interest was obtained from AccuStandard (New Haven, CT, USA) and diluted for a standard curve. Target compounds were quantified using selective ion monitoring.

3.2.2.2 Quality assurance

For the purpose of evaluating our preliminary results, method detection limits (MDLs) and quantification limits (QLs) were adopted from a previous study on Class A biosolids (Chapter 2, Section 2.2.4). All samples had concentrations of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-209 above QLs, while 8 of 9 samples had concentrations of BDE-183 above QLs. 2 samples had concentrations of BDE-47 and BDE-99 out of range of the standard curve's upper limit. These concentrations were not included in analysis since they were greater than

10% above the most concentrated standard. PCB-209 recoveries averaged 99.90±2.27% (n=2) in sand and 80.82±27.92% (n=9) in matrix samples. The batch of samples included a blank, sand and biosolids spiked with a BDE-Mix (approximately 50 ng of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 and 400 ng of BDE-209, Cambridge Isotope Laboratories, Inc., Andover, MA and (AccuStandard, New Haven, CT, USA), and a laboratory duplicate. The blank showed no contamination by any of the PBDE congeners. Spiked sand and biosolids PBDE recovery are given in Table 4. Recoveries for this single spiked biosolids sample were quite high, especially in congeners BDE-47, BDE-99, and BDE-209. The PCB-209 recovery for the spiked biosolids sample was also high (165.9%). Future analyses will include additional spiked matrix samples and provide a more accurate assessment of any matrix effects occurring within our samples. Results shown here were not corrected for recovery. The batch duplicate had an average difference of 4.622±3.849% (n=7) for all PBDE congeners except BDE-183, which was below MDL for one of the duplicates and could not be determined. Figures and statistical analysis were done using GraphPad Prism 6 software (GraphPad Software, Inc., San Diego, CA, USA).

Table 4. Recoveries in spiked sand and biosolids samples.

	PCB-209	***BDE-28***	***BDE-47***	***BDE-99***	***BDE-100***	***BDE-153***	***BDE-154***	***BDE-183***	***BDE-209***
Spiked Sand	102.2%	86.09%	88.35%	93.93%	96.14%	86.81%	87.53%	84.23%	107.5%
Spiked Biosolids	165.9%	132.8%	275.7%	275.7%	128.23%	140.9%	139.3%	160.0%	236.4%

3.2.3 Polymerase Chain Reaction (PCR) and Quantitative Polymerase Chain Reaction (qPCR) for organohalide-respiring bacteria

DNA extraction was performed according to the manufacturer's protocol on 0.10-0.15 g of sludge per sample using the QIAGEN QIAamp PowerFecal Pro DNA Kit. Purity of extracted DNA was confirmed via spectroscopy using a NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific). Polymerase chain reaction (PCR) and quantitative Polymerase Chain Reaction (qPCR) were performed on extracted genomic DNA of organohalide respiring bacteria (OHRB). Primers targeting the organohalide respiring phylogenies of *Dehalogenimonas* and *Dehalobacter* are described in Smith et al. (2015) and were purchased from Integrated DNA Technologies. Thermocycling protocol for PCR of *Dehalobacter* was 94℃ for 3 minutes followed by 45 cycles of 94℃ for 30 seconds, 60℃ for 30 seconds, and 72℃ for 15 seconds. Thermocycling protocol for PCR of *Dehalogenimonas* was 94℃ for 15 minutes followed by 45 cycles of 94℃ for 30 seconds, 56℃ for 30 seconds, and 72℃ for 15 seconds. Results were visualized using gel electrophoresis that included a positive control (extracted DNA from an SDC-9 culture for *Dehalobacter* and WBC-2 for *Dehalogenimonas*).

Reaction conditions were optimized for qPCR in order to best quantify targeted organohalide respiring bacteria in samples. Each qPCR reaction totaled 25 uL and included 12.5 uL of SYBR Green (iTaq™ Universal SYBR® Green Supermix), 0.25 uL of each primer, and 2 uL of sample DNA. All qPCR analyses were conducted on a CFX Connect Real Time System with CFX Manager software (Bio-Rad Laboratories). Thermocycling protocol for *Dehalogenimonas* was 94℃ for

2 minutes and 10 seconds followed by 45 cycles of 94℃ for 30 seconds, 60℃ for 20 seconds, and 72℃ for 15 seconds. Melt curve analysis was performed to ensure specific amplification of primers and that contribution by primer dimer was not significant. Standards were prepared from extracted WBC-2 DNA and had an R^2 of 0.9985. Reaction efficiency was 106.7%. Each qPCR analysis was performed in triplicate and included a positive control (extracted DNA from an WBC-2 culture), a negative control (E. coli), and blanks (qPCR reaction with no template DNA and just PCR Water). Melt curve analysis revealed no primer hits for the negative controls, and five of the six blank replicates (one replicate with qPCR reagents showed a positive hit). While positive primer hits were identified in each sample, four individual replicates out of 48 total did not have positive hits and were excluded from analysis. One sample replicate was found to have no distinct phases of amplification during qPCR cycles, so it could not be quantified.

3.3. Results and discussion

3.3.1 PBDE Analysis

3.3.1.1 Overview of findings

Preliminary results of PBDE concentrations presented here represent biosolid produced after the final step of THP-AnF's, AnF1's, and AnF2's treatment trains. PBDEs were measured in samples collected during July and August 2019. The average total solids content of these samples were 29.65±7.54% (n=16). Samples from AeF were excluded from the PBDE analysis presented here, since concentrations of BDE-47 and BDE-99 were found to be greater than the upper limits

of the standard curve. In future analysis, the extracts if these samples can be diluted to within range of the standard curve so concentrations can be quantified.

On average, the congeners BDE-47, BDE-99, and BDE-209 contributed to 89.57±3.71% (n=7) of the total PBDEs detected in biosolids from THP-AnF, AnF1, and AnF2 (Appendix B). Commercial penta-BDE formulas consist primarily of BDE-47 and BDE-99, while octa-BDE and deca-BDE formulas consist mainly of BDE-209. Due to their widespread use worldwide, these congeners are commonly reported to be the most abundant PBDEs found in biosolids (Kim et al., 2013; Davis et al., 2015; McGrath et al., 2020). These PBDE congeners are also all subject to further reductive debromination during anaerobic sludge treatment (Chang et al., 2016). Chang et al. (2016) indicated several pathways for which BDE-209 can debrominate to smaller PBDE congeners.

Concentrations of PBDEs ranged widely between the three AnD facilities sampled in this study (Figure 6). Total PBDEs (sum of all congeners measured in this study) ranged from 385.8 to 1470 ug/kg d.w. with a median of 1126 ug/kg d.w (n=9). This was generally lower than total PBDEs quantified in other North American facilities (Davis et al., 2015; Kim et al., 2013; Hale et al., 2012). Concentrations of BDE-209 ranged from 123.8 to 1031 ug/kg d.w. with a median of 471.4 ug/kg d.w. (n=9). The 2009 Targeted National Sewage Sludge Survey (TNSSS) sampled biosolids from 74 WWTPs in the US that were statistically representative of 3,337 treatments plants in US. Interestingly, average BDE-209 concentrations (2181±3463 ug/kg d.w.) in final biosolids reported by the TNSSS survey were between 2.5-13.6 times greater than in our study. BDE-47+BDE-99 varied less between samples and

had an average concentration of 305.3±75.7 ug/kg d.w. (n=9) in biosolids from the three WWTPs. Levels of these congeners were much lower than concentrations reported by TNSSS (1426±748 ug/kg d.w.) but were in the same order of magnitude of other North American plants (Gorgy et al., 2013) and in final biosolids presented previously in this study (Chapter 2, Section 3.3.1).

Two samples of final biosolids were collected at each location to gauge the variability in PBDE concentrations in solids at each WWTP (Table 5). In general, concentrations of BDE-209 tended to be more variable at each location than concentrations of other congeners measured in this study.

Table 5. Percent difference of PBDE congener concentrations in final biosolids samples collected in duplicate from three different Mid-Atlantic WWTPs.

Location	***Average of Smaller PBDE Congeners[a]***	***BDE-209***
THP-AnF	17.72 ± 6.44%	47.36%
AnF1	5.012 ± 4.031%	34.46%
AnF2	13.55 ± 5.17%	53.89%

[a]Smaller PBDE congeners include BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183.

3.3.1.2 Concentrations and congener distributions of PBDEs in biosolids

Average concentrations of BDE-47+BDE-99, BDE-209, and total PBDEs (all congeners measured in this study) in biosolids collected from the three anaerobic facilities are shown in Figure 5. AnF2 was found to have the greatest load of total PBDEs in biosolids, followed by loads in biosolids produced at AnF1 (average of 1298±172 ug/kg d.w. (n=2) and 1225±116 ug/kg d.w. (n=2), respectively). Total PBDEs in biosolids from THP-AnF (averaging 442.6±68.3 ug/kg d.w. (n=3)) were

64.92% less than the average loads at AnF1 and AnF2. The lower levels of total PBDEs in THP-AnF biosolids may be due to more partial debromination of PBDEs to congeners not measured in this study, or to more complete debromination of PBDEs compared to other facilities. Different incoming loads of PBDEs in sludge may also contribute to different total loads in biosoldis. The THP-AnF solids treatment train is unique compared to AnF1 and AnF2 in that both physical and biological debromination may be possible during the course of treatment. Previous studies of THP-AnF have provided evidence of the thermal degradation of chlorinated organic compounds during THP pretreatment of sludge (Armstrong et al., 2017b). However, studies have shown that PBDE loads in biosolids can vary greatly between regions (Kim et al., 2013; McGrath et al., 2020). Mass balances of total PBDEs across facilities' treatment trains will be utilized in future analysis to more accurately assess if evidence of debromination is present in samples from this study.

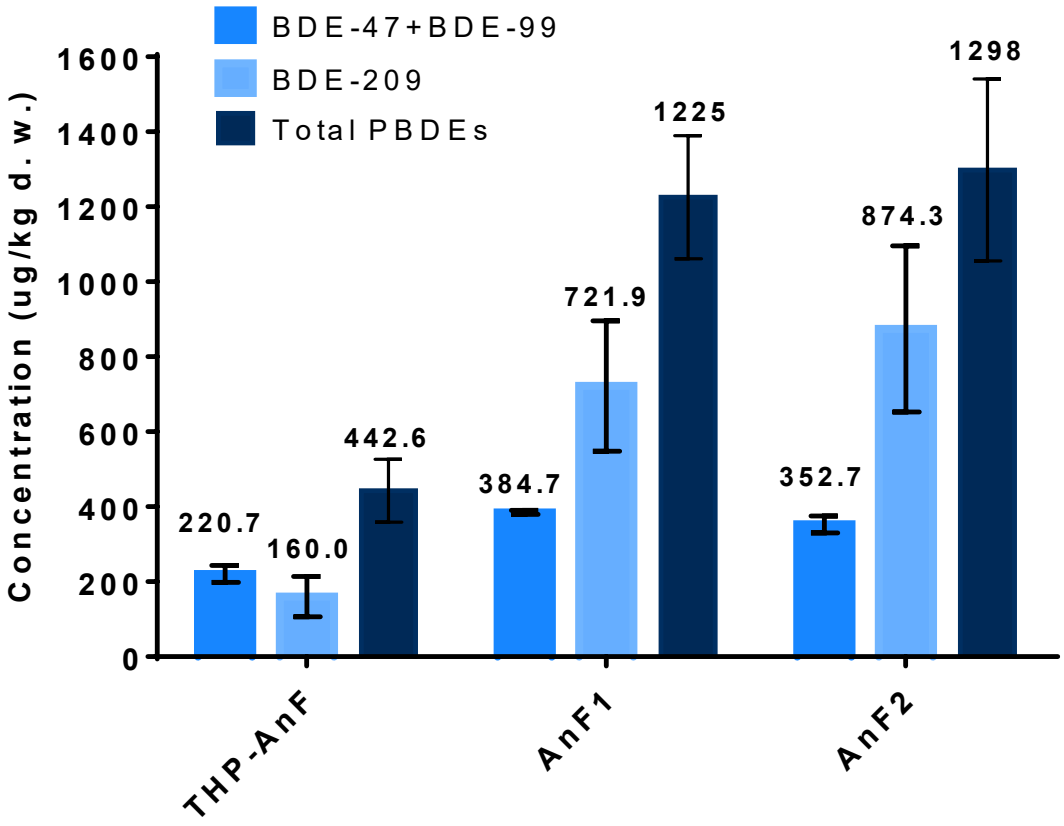


Figure 5. Average concentrations of BDE-47+BDE-99, BDE-209, and total PBDEs in final biosolids collected from three different Mid-Atlantic WWTPs. Total PBDEs include all congeners measured in this study. Average concentrations are provided above each bar (n=3 for THP-AnF and n=2 for both AnF1 and AnF2).

BDE-209 accounts for the majority of PBDE concentrations in biosolids collected from AnF1 and AnF2. BDE-209 is often reported to be the dominant congener in PBDE-contaminated biosolids and sludge (Gallen et al., 2016; Hale et al., 2012) since deca-BDE formulations account for the largest fraction of PBDEs used commercially. In 2001, it was estimated that deca-BDE accounted for 83.3% of the

PBDE market demand globally (La Guardia et al., 2006). Results in Figure 5 indicate that commercial formulas of deca-BDE and octa-BDE are the two main contributors to concentrations of PBDEs in biosolids at AnF1 and AnF2.

To investigate possible degradation products present in biosolids from these three facilities, the ratios of congener concentrations were compared (Figure 6). Concentrations were first normalized to account only for the diphenyl ether ring of each congener. This allows meaningful comparisons to be made without the molecular weight of congeners skewing concentration values. A one-way ANOVA was conducted to compare the congener ratios in Figure 6 between the three facilities. The simple effects comparison revealed the ratio in THP-AnF biosolids was significantly greater than ratios in biosolids from AnF1 or AnF2 ($p \leq 0.05$), while congener ratios in AnF1 and AnF2 were not significantly different from one another. A one-sample t-test showed that ratios in AnF1 and AnF2 (means 1.217±0.317 (n=2) and 0.8420±0.174 (n=2), respectively) were not significantly different from 1. This indicates that the number of smaller PBDE congeners were generally equal to the number of BDE-209 molecules. Smaller congeners include all PBDEs measured in this study besides BDE-209. The congener ratio of THP-AnF biosolids revealed that there was approximately 3 times the number of smaller PBDE congeners in biosolids than BDE-209 molecules. Considering deca-BDE accounts for large majority of commercial PBDE mixes used (La Guardia et al., 2006), its low presence in THP-AnF biosolids compared to smaller congeners may indicate more efficient degradation of this congener during THP-AnD solids treatment compared to degradation in non-THP AnD systems in this study.

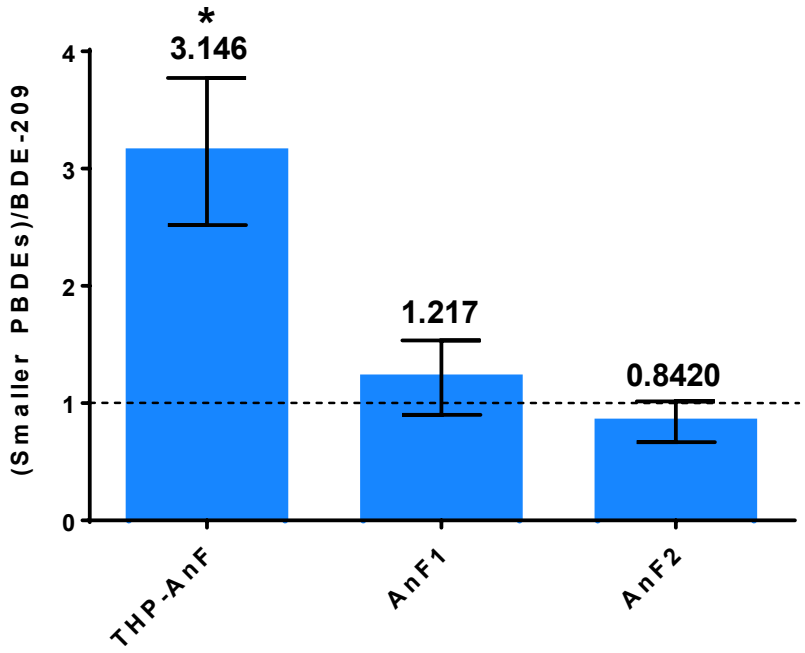


Figure 6. Average ratio of the sum of smaller PBDE congeners to BDE-209 measured in biosolids from three different Mid-Atlantic WWTPs. Smaller PBDE congeners include BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183. Concentrations were first normalized to account only for the diphenyl ether ring of each congener. Dotted line indicates a ratio of 1, where the average normalized concentration of smaller PBDEs is equal to that of BDE-209. One-way ANOVA was run with simple comparison tests to compare the congener ratio means. An asterisk indicates a significant difference, where $p \leq 0.05$.

The comparison of ratios of PBDE congeners in biosolids to ratios found in commercial formulations that enter wastewater inlet streams can also help assess if degradation may be occurring during solids treatment (La Guardia et al., 2007; Knoth

et al., 2007). This comparison was performed between the ratio of congeners commonly found in penta-BDE formulations (hereon referred to as "pentaBDEs") in biosolids samples collected during this study and commercial mixes.

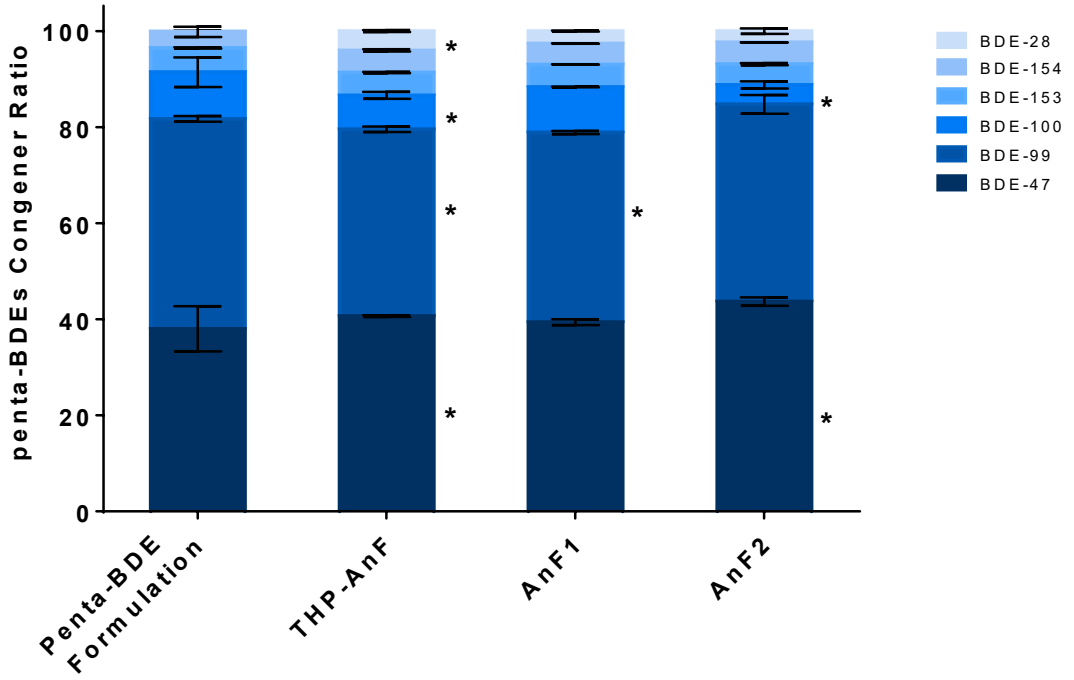


Figure 7. Congener contribution of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 to the sum of these 5 congeners found in commercial penta-BDE formulas (La Guardia et al., 2017) and final biosolids from 3 different Mid-Atlantic WWTPs. A two-way ANOVA with simple effects comparison was conducted to evaluate the differences in congener contributions between penta-BDE formulations and biosolids from the three sampling locations. Asterisks indicate a statistically significant difference ($p \leq 0.05$) between each WWTP's mean ratio and the mean of the penta-BDE formulation's ratio.

A two-way ANOVA was run to compare the ratio of penta-BDE congeners between biosolids samples and commercial mixes. Results of the simple effects comparison tests are shown in Figure 7. The ratios of penta-BDE congeners were statistically significantly different to varying degrees in biosolids collected from the three WWTPs compared to the ratios in commercial penta-BDE formulas. Results indicate that larger PBDE congeners may be degrading to smaller ones during wastewater treatment at each of these WWTPs, leading to altered congener ratios. Ratios of penta-BDEs in biosolids from THP-AnF deviated the most, with 4 of the 6 congeners showing significant differences from ratios in commercial formulations. This may indicate increased levels of PBDE degradation occurring in the THP-AnF treatment train compared to WWTPs without THP pretreatment. However, ratios of penta-BDEs in biosolids must be compared to levels in the influent sludge to determine if degradation is truly occurring during the treatment processes.

Examining congener ratios in biosolids alone does not definitively indicate if degradation of PBDEs has occurred during solids treatment. In future analysis, congener ratios will be compared in samples collected along the treatment train. Comparing ratios between influent sludge and final biosolids can reveal if degradation is occurring during treatment. Step-to-step comparisons for each part of the solids treatment train will indicate at which step degradation is occurring, and may indicate whether debromination was through physical or biological processes. A mass balance of normalized PBDE concentrations along each step of solids treatment will be utilized to determine if complete mineralization of congeners occurs during

degradation. This future work will help answer the central questions of this study, which include i) do THP-AnD treatment systems lead to more efficient degradation of PBDEs in sludge than AnD systems without THP pretreatment, through either physical or biological debromination? and ii) where along the treatment train does degradation occur?

3.3.2 Organohalide-respiring bacteria in WWTPs' solids treatment trains

3.3.2.1 Primer survey of halorespiring bacteria

Sludge samples were screened for the presence of two obligate organohalide-respiring bacteria (OHRB) phylogenies and one facultative phylogeny. *Dehalobacter* and *Dehalogenimonas* have been shown to be present in sludge (Smith et al., 2016) and degrade PBDEs (Robrock et al., 2008; Chen et al., 2018). Primers for each phylogeny were used in PCR and results were visualized by gel electrophoresis. Two obligate bacteria, *Dehalobacter* and *Dehalogenimonas*, were confirmed present in the solids treatment train of all four WWTPs (Table 6). *Dehalogenimonas* was detected in almost every sample and was selected for further analysis by qPCR. *Dehalobacter* was found more sparingly and was present in 3 of the 4 digester systems sampled. Obligate OHRB account for a very small percent of anaerobic bacteria present in AnDs (Nelson et al., 2011). Although they exclusively use halogenated organics as a source of energy, they are likely only able to produce low concentrations of degraded contaminants (Krzmarzick and Novak, 2014). Interestingly, DB and *Dehalogenimonas* were both detected in the aerobic digester sampled in this study.

Table 6. Presence of *Dehalobacter* and *Dehalogenimonas* in sludge samples collected from the solids treatment train of four different Mid-Atlantic WWTPs. A (++) indicates strong presence, (+) indicates a faint presence, and (-) indicates no presence in each sample.

WWTP	***Sample Location***	***Presence of Dehalobacter***	***Presence of Dehalogenimonas***
THP-AnF	THP influent	++	++
	THP effluent	-	-
	AnD effluent	++	++
	Final biosolids	-	++
AnF1	Influent	++	++
	Primary AnD	++	++
	Secondary AnD	++	++
	Final biosolids	-	++
AnF2	Thickened influent	-	++
	Fermented influent	++	++
	AnD effluent	-	++
	Centrifuged final biosolids	-	-
	Belt pressed final biosolids	-	++
AeF	AnD influent	+	++
	AnD effluent	+	++
	Final biosolids	-	-

3.3.2.2 Abundance of *Dehalogenimonas* in solids treatment trains

Abundances of the obligate OHRB *Chloroflexi Dehalogenimonas* was quantified in sludge samples collected from 4 different WWTPs (Figure 8). Abundances ranged from 6.02×10^{5} to 3.14×10^{11} gene copies/g d.w. and had a median of 1.13×10^{9} gene copies/g d.w (n=41). Fischer (2019) reported similar

abundances of *Dehalogenimonas* in digester sludge from THP-AnF at an average of 10^{10} gene copies/gram d.w. However, abundances of *Dehalogenimonas* in this study were generally higher than abundances of OHRBs found in previous studies of AnDs (Smith et al., 2015, ~10^7 gene copies/g d.w.) and other bioreactors (Zhao et al., 2020 ~10^7 gene copies/g d.w.). Absolute abundances will therefore be reassessed. Relative abundances between samples in this study are not expected to change, however.

Abundances in facilities' influent streams varied widely from 5.82×10^7 to 1.15×10^{10} gene copies/g d.w. (median 1.13×10^9 gene copies/g d.w. (n=12)). Anaerobic bacteria are known to be present and active in sewer systems. Biofilms found on sewage pipe surfaces are capable of creating anaerobic conditions that support this activity (Nielsen and Hvitved-Jacobsen, 1988). A more recent study by Krumins et al. (2018) reported the OHRB *Dehalococcoides* in abundances as high as 6.6×10^4 gene copies/g d.w. in U.S. city sewer sediments. Microcosms inoculated with sewer sediments showed significant reduction of polychlorinated biphenyls (Krumins et al., 2018), indicating that OHRB may be capable of organohalide reduction in sewers. *Dehalogenimonas* may be abundant in influent streams in this study due to its presence and activity in sewer systems. The approximate 10^5 decrease in abundance from THP influent to effluent (Figure 8) was expected, as THP treatment utilizes high pressure and heat to hydrolyze macromolecules. Tong et al. (2019) also reported a reduction in microbial biomass and a shift to spore-forming bacteria in sludge following full-scale THP treatment.

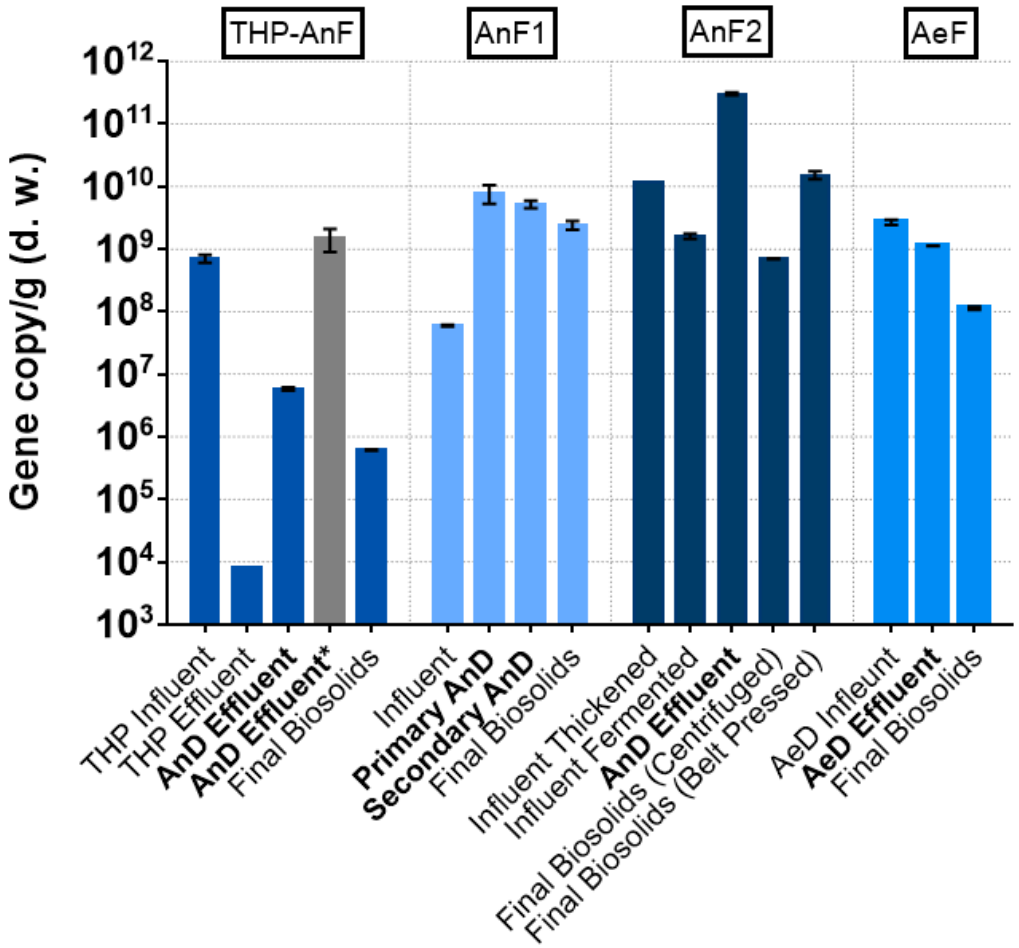


Figure 8. Average abundance of *Dehalogenimonas* in sludge samples collected from the solids treatment trains of four different Mid-Atlantic WWTPs. Bar shown in gray represents the average abundance of *Chloroflexi Dehalogenimonas* in sludge collected in July 2016 by Fischer (2019) from a THP-AnF digester and is included for comparison with current samples collected for this study.

Abundance of *Dehalogenimonas* varied widely in digesters from 5.56×10^{6} to 3.14×10^{11} gene copies/g d.w. with a median of 5.60×10^{9} gene copies/g d.w. (n=15). The highest abundance was found in the AnD sludge of AnF2, which had an average

abundance of $3.04\times10^{11} \pm 1.21\times10^{10}$ gene copies/g d.w. (n=3). The lowest abundance in digesters was found in samples from THP-AnF. This facility recently experienced irregularities in operations in early 2019 (about a year before our samples were collected) that may have led to low abundance of *Dehalogenimonas* in its digester. An increase in fecal coliforms in the AnD effluent of one of the four digesters led to the discovery of a leak in the digester's coolant system. This likely disrupted the activity of the digester community by decimating organism populations, including *Dehalogenimonas*. The four digesters had periodically been run in series, spreading the coolant leak to other digesters. High fecal coliforms were found in the digester effluent as recently as November of 2019 (about 5 months before our samples were collected), and this irregularity likely influenced our composite sample collected in March 2020. For this reason, a historical sample collected in July 2016 from one of the four digesters was included in our analysis and is shown in gray in Figure 8. This sample is more representative of the abundance of *Dehalogenimonas* in digesters at this location since it was sampled during normal operating conditions. Abundance of *Dehalogenimonas* in historical digester sludge was approximately 10^3 times greater than in sludge sampled recently in this study and is similar to the abundances found in the other facilities' digesters (Figure 8).

Anaerobic digesters provide ideal conditions for OHRB to reductively dehalogenate organic compounds since this process occurs in the absence of oxygen. Therefore, we expect PBDE degradation to predominantly occur in AnDs sampled in this study. Biosolids samples collected from THP-AnF showed the highest ratio of smaller PBDEs to BDE-209 (Figure 6), possibly indicating more efficient PBDE

degradation occurring in this facility compared to AnF1 and AnF2. However, digester sludge from THP-AnF showed the lowest abundance of *Dehalogenimonas* of all digesters samples in this study. While grab samples used for PBDE analysis and qPCR were collected approximately 8 months apart, both were collected after the suspected digester coolant leak described previously. If more efficient debromination is occurring during THP-AnF's solids treatment, it may be largely due to physical degradation occurring during THP pretreatment rather than biological debromination during anaerobic digestion. Future analysis will re-quantify PBDEs in sludge collected from THP-AnF during the same sampling date as samples used in PCR/qPCR analysis.

Connections between PBDE concentrations and abundance of *Dehalogenimonas* in samples from AnF1 and AnF2 were mixed. While the greatest abundance of *Dehalogenimonas* in AnD samples was found in AnF2 samples, this facility produced biosolids with the lowest smaller PBDEs-to-BDE-209 congener ratio (Figure 6). The highly significant differences ($p \leq 0.05$) between penta-BDE congeners in AnF2 biosolids and commercial formulations (Figure 7) may be due to biological debromination occurring in its digester. Abundances of *Dehalogenimonas* in AnF1 digester sludge were approximately 10^2 less than that of AnF2. Penta-BDE ratios in biosolids produced from this facility were generally not significantly different than those found in commercial formulas (Figure 7), indicating that the digesters-in-series system used at this facility experience less efficient PBDE degradation during solids treatment compared to other treatment trains in this study. Future work will include a complete dataset of PBDE quantification in sludge

samples from each treatment train step. Comparing PBDE concentrations with *Dehalogenimonas* abundance in these samples will aid in assessing where biological PBDE degradation is likely occurring.

Dehalogenimonas was detected in the aerobic digester of AeF as well. There is no evidence that obligate OHRB such as *Dehalogenimonas* can degrade PBDEs aerobically. However, insufficient mixing of aerobic digesters can lead to the formation of localized anaerobic environments in the digester. The presence of OHRB in aerobic bioreactors has been identified in other studies. Zhao et al. (2020) showed that the abundance of *Dehalogenimonas* was the same order of magnitude in sludge from anoxic and swing (mix of aerobic and anaerobic) zones in a municipal swing zone tank bioreactor. Fischer (2019) also found similar levels of *Dehalogenimonas* in aerobic THP-AnF reactors on the water side of wastewater treatment. Since halogenated organic compounds generally have a large K_{ow} and partition to solid organic matter in wastewater streams, *Dehalogenimonas* in these reactors are likely found among solids in these reactors. Localized anaerobic environments in AeF's digester may make the biological degradation of low PBDE concentrations possible. Future comparison with sludge samples at each step of AeF's solid treatment will help determine if debromination is occurring.

To evaluate if the addition of THP pretreatment to AnD leads to an increase in OHRB in the digester community, the abundances of each facility's digester influent and effluent were compared (Figure 9). THP influent was used for THP-AnF calculations so that the analysis would not be affected by the significant decrease seen in the abundance in THP effluent samples. The historical digester sample was used in

this analysis to account for the impaired operations THP-AnF experienced during our sampling period. For AnF1 calculations, only the primary digester was considered since it directly receives the influent stream. AnF2 calculations included the average of the two influent streams, since they are combined before feeding into the AnD. Each anaerobic digester facility experienced an increase in abundance between digester influent and effluent, while AeF experienced a decrease in abundance. Increases in abundance are reflective of the ideal environment in AnDs for reductive dehalogenation. AeF's decreased abundance likely indicates poor conditions for OHRB activity in any anaerobic pockets that may be present in the digester.

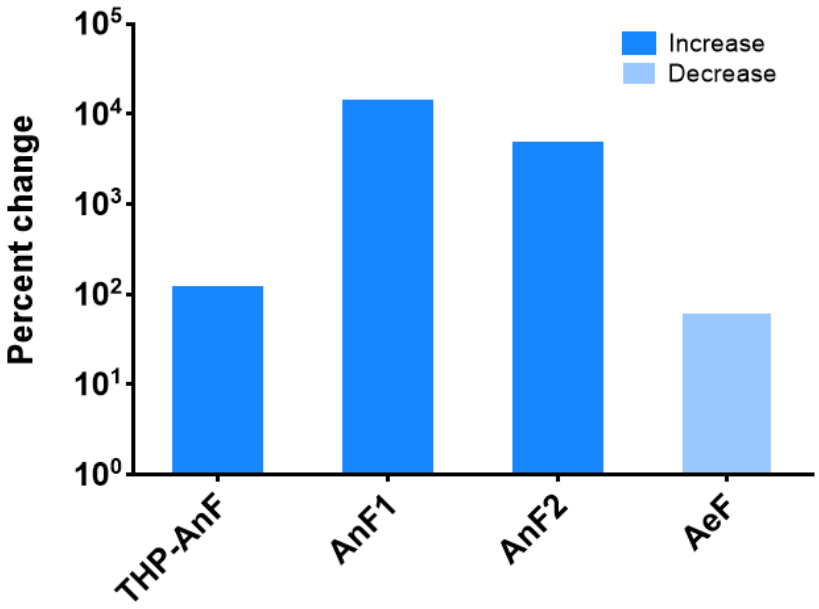


Figure 9. The percent change in *Dehalogenimonas* abundance from digester influent to digester effluent sludge collected from four different Mid-Atlantic WWTPs.

AnD sludge pretreated with THP did not have the greatest increase in *Dehalogenimonas* abundance. This may indicate that THP pretreated sludge does not better support the growth of dehalogenating microbes in AnDs compared to AnDs fed with non-THP treated sludge. A similar conclusion was reached by Fischer in her 2019 study of bench-scale AnD mesocosms inoculated with THP and non-THP pretreated sludge from THP-AnF. This study concluded that there were no significant differences between the microbial communities of sludge under the two treatment conditions. Analysis presented in this study is not ideal since a THP-AnF digester effluent sample collected at the same time as the THP influent could not be included. There may be differences in the composition of the THP influent between 2017 and 2020 that result in unrelated concentrations of *Dehalogenimonas*. Therefore, this analysis should not be considered as definitive evidence and should be repeated in the future at another THP-AnD facility or after the digester community fully recovers at THP-AnF.

3.4 Conclusions

It is important to understand how PBDEs are transformed during full-scale municipal solids treatment, since biosolids can leach toxic degradation products into the environment after land application. Different treatment system strategies may cause varying degrees of debromination to occur. Results of this study show that biosolids produced at the THP-AnD facility had a higher ratio of small PBDE congeners to BDE-209 (~3:1 ratio) than biosolids produced at other facilities in this study. Penta-BDE congener ratios in THP-AnF final biosolids were most significantly

different from ratios in commercial formulations as compared to other facilities' biosolids. While our findings seem to indicate more efficient debromination occurs at THP-AnF, AnD at this facility did not have the highest abundances of the OHRB *Dehalogenimonas.* Results of this study do not suggest that THP pretreatment of sludge increases *Dehalogenimonas* abundance in anaerobic digesters. If more efficient debromination is occurring during THP-AnF's solids treatment, it may be largely due to physical degradation occurring during THP pretreatment rather than biological debromination during anaerobic digestion. Examining congener ratios in biosolids alone does not definitively indicate if degradation of PBDEs has occurred during solids treatment. In future analysis, congener ratios will be compared in samples collected along the treatment train. Step-to-step comparisons of PBDE concentrations and OHRB abundances for each part of the solids treatment train will indicate at which point degradation is occurring, and may indicate whether it is due to physical or biological processes.

Chapter 4: Conclusions

While the use of nutrient-rich biosolids for soil amendment has many benefits, biosolids are known to be contaminated by persistent organic pollutants like PBDEs. Land application of contaminated biosolids has raised a pressing environmental concern. Despite their phase-out from importation and manufacturing in the U.S. during the early 2000s, PBDEs have continued to be present in WWTPs and detected in final biosolids. This study analyzed temporal trends of PBDEs in Class A and Class B biosolids before and after a change in solids treatment at a Mid-Atlantic WWTP. This study showed that the implementation of THP-AnD treatment system in 2014 at the target WWTP produced biosolids with less total amount of PBDEs, but with a higher fraction of smaller, more toxic congeners than found in lime-stabilized biosolids previously produced at this WWTP. Congener distributions indicated that increased degradation of PBDEs occurred during THP-AnD treatment compared to lime stabilization, as the distribution shifted to favor a majority of lesser-brominated PBDEs in THP-AnD treated biosolids compared to distributions in lime-stabilized biosolids that favored BDE-209. Results also indicated that BDE-209 concentrations in biosolids produced at the target WWTP were unaffected by the phase-out of deca-BDEs from commercial markets, while the concentrations of penta-BDEs appear to have stabilized after previously decreasing following their early 2000s phase-out.

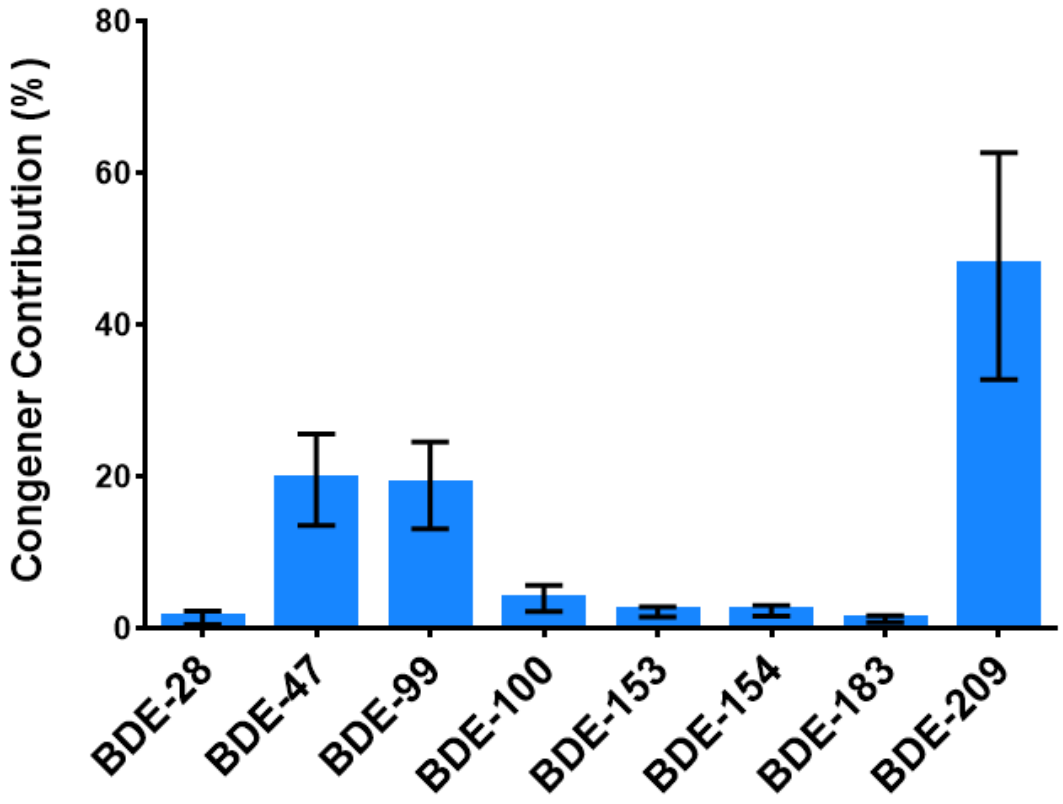
Different municipal solids waste treatment strategies may be capable of transforming PBDEs to varying degrees. THP pretreatment to anaerobic digestion has

been shown increase digestion of solid waste and produce high-quality biosolids. However, the effects of THP-AnD on PBDE degradation and the growth of debrominating bacteria are not well understood. Results of this study show that biosolids produced at the THP-AnD facility had a higher ratio of small PBDE congeners to BDE-209 (~3:1 ratio) than biosolids produced at other facilities in this study. Penta-BDE congener ratios in THP-AnF final biosolids were most significantly different from ratios in commercial formulations as compared to other facilities' biosolids. While our findings seem to indicate more efficient debromination occurs at THP-AnF, AnD at this facility did not have the highest abundances of the OHRB *Dehalogenimonas*. Evidence of more efficient debromination is occurring during THP-AnF's solids treatment, it may be largely due to physical degradation occurring during THP pretreatment rather than biological debromination during anaerobic digestion. Results of this study do not suggest that THP pretreatment of sludge increases *Dehalogenimonas* abundance in anaerobic digesters. Future analysis will examine PBDE concentrations and OHRB abundance in each step of the solids treatment train of the four WWTPs. This will help elucidate which steps of treatment lead to degradation and if physical or biological processes are responsible.

Appendices

Appendix A. Recovery of the surrogate PCB-209 in Class B biosolids samples

Order of GC-MSD Analysis	Sample ID	Surrogate (PCB-209) Recovery
1	Sand Blank	106.8%
2	Biosolids 2/23/12	111.7%
3	Biosolids 6/25/12	88.70%
4	Biosolids 12/17/12	84.99%
5	Biosolids 3/22/13	72.89%
6	Biosolids 8/30/13	79.15%
7	Biosolids 10/30/13	78.06%
8	Biosolids 1/16/14	38.18%
9	Biosolids 5/7/14	72.14%
10	Biosolids 11/19/14	33.05%
11	Sand Spike	67.00%
12	Matrix Spike	5.502%



Appendix B. Average contribution of each congener to the concentration of total PBDEs measured in biosolids collected from three Mid-Atlantic WWTPs.

Appendix C. Concentrations of PBDEs (ug/kg d.w.) in Class B biosolids after correction for PCB-209 recovery.

Sample Name	PCB-209 Recovery	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 209	Weight (g)	Total Solids Content
2/23/2012	111.7%	0.626174	100.6109	25.03004	117.6022	7.547935	8.64797	-0.44001	333.4461	1.5408	0.343288
6/25/2012	88.70%	0.742693	103.6064	21.72993	107.1434	6.701754	8.258727	-0.79541	380.5615	1.4928	0.355885
12/17/2012	84.99%	1.421725	79.49025	18.00673	85.44736	5.026315	5.923267	-1.25235	524.2769	1.5947	0.43076
3/22/2013	72.89%	32.67338	70.13149	16.80516	77.69634	4.670919	5.178628	-1.48928	304.9129	1.5429	0.298804
8/30/2013	79.15%	0.102418	64.19129	14.50354	67.96526	4.146287	4.755538	-1.33697	405.1176	1.5282	0.403607
10/30/2013	78.06%	0.261686	72.55156	16.33129	76.46092	4.366294	5.398635	-1.13388	358.6961	1.5673	0.374805
1/16/2014	38.18%	-1.36595	35.67499	8.952596	38.85663	1.743133	2.216994	-1.84467	264.0931	1.5216	0.352804
5/7/2014	72.14%	-0.05155	70.06379	15.65435	74.19315	4.281676	5.297093	-1.23542	343.3126	1.54	0.349223
11/19/2014	33.05%	0	23.96385	6.210969	24.7931	0.473861	0.761563	0	195.6371	1.5614	0.33632

Appendix D. Concentrations of PBDEs (ug/kg d.w.) in biosolids collected from 4 Mid-Atlantic WWTPs.

	PCB-209 Recovery	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 209	Weight (g)	Total Solids Content
AeF Sample 1	108.9%	7.408	298.1	78.06	345.8	28.52	34.31	16.39	456.1	1.699	0.4601
AeF Sample 2	104.6%	8.938	309.6	74.46	341.1	29.91	35.43	17.82	471.4	1.743	0.3222
AnF2 Sample 1	96.55%	8.370	185.6	15.19	183.0	19.62	19.29	7.796	1030.9	1.707	0.3674
AnF2 Sample 1	86.48%	11.01	177.7	18.35	159.1	18.17	16.69	7.681	717.7	1.495	0.3720
AnF1 Sample 1	91.35%	12.17	191.8	45.43	189.1	20.73	22.44	14.62	845.0	1.738	0.2090
AnF1 Sample 1	91.75%	13.24	192.3	47.17	196.2	21.61	23.24	16.77	598.8	1.706	0.2121
THP-AnD Sample 1	90.33%	11.83	125.8	23.53	119.6	13.44	14.16	8.577	221.6	1.497	0.3363
THP-AnD Sample 2	72.20%	10.79	103.2	18.60	97.86	12.22	12.28	7.053	123.8	1.547	0.3355
THP-AnD Sample 3	86.80%	10.69	109.5	16.82	106.2	12.68	12.92	0.000	134.5	1.497	0.3355

Appendix E. Abundance of *Dehalogenimonas* in sludge samples collected from 4 Mid-Atlantic WWTPs.

Sample	Mass Sample extracted for DNA (g wet weight)	Dilution Factor of Template DNA (diluted conc/undiluted conc.)	Total Solids Content of Biosolids sample	Gene copy per g (dry weight)
AeF influent	0.186	0.082	0.022394	2.77E+08
	0.186	0.082	0.022394	2.86E+09
	0.186	0.082	0.022394	2.51E+09
AeF AeD effluent	0.169	0.586	0.008894	1.12E+09
	0.169	0.586	0.008894	1.13E+09
	0.169	0.586	0.008894	1.13E+09
AnF2 thickened influent	0.131	0.093	0.057432	1.15E+10
	0.131	0.093	0.057432	1.31E+10
	0.131	0.093	0.057432	2.64E+10
AnF2 fermented influent	0.147	NA	0.053948	1.73E+09
	0.147	NA	0.053948	1.66E+09
	0.147	NA	0.053948	1.45E+09
AnF1 AnD effluent	0.112	0.398	0.030125	3.14E+11
	0.112	0.398	0.030125	3.1E+11
	0.112	0.398	0.030125	2.87E+11
AeF final biosolids	0.111	0.096	0.348256	1.17E+08
	0.111	0.096	0.348256	1.17E+08
	0.111	0.096	0.348256	1.07E+08
AnF2 centrifuged final biosolids	0.175	NA	0.298517	6.95E+08
	0.175	NA	0.298517	7.12E+08
	0.175	NA	0.298517	6.93E+08
AnF2 belt pressed final biosolids	0.168	0.413	0.332109	1.59E+10
	0.168	0.413	0.332109	1.28E+10
	0.168	0.413	0.332109	1.7E+10
AnF1 influent	0.131	NA	0.06873	59471257
	0.131	NA	0.06873	61036290
	0.131	NA	0.06873	58219230
AnF1 Primary AnD effluent	0.112	0.071	0.079557	1.09E+10
	0.112	0.071	0.079557	6.62E+09
	0.112	0.071	0.079557	6.17E+09
AnF1 Secondary AnD effluent	0.166	0.215	0.034289	5.6E+09
	0.166	0.215	0.034289	4.38E+09
	0.166	0.215	0.034289	5.62E+09
AnF1 final biosolids	0.121	0.101	0.207298	5.85E+14
	0.121	0.101	0.207298	2.15E+09

	0.121	0.101	0.207298	2.7E+09
THP-AnD THP influent	0.127	0.066	0.058699	8.15E+08
	0.127	0.066	0.058699	6.85E+08
	0.127	0.066	0.058699	6.23E+08
THP-AnD THP effluent	0.125	NA	0.159885	102.2065
	0.125	NA	0.159885	7929.088
	0.125	NA	0.159885	802.7499
THP-AnD AnD effluent	0.133	NA	0.071781	6203739
	0.133	NA	0.071781	5556904
	0.133	NA	0.071781	5645109
THP-AnD final biosolids	0.142	NA	0.298935	619490.3
	0.142	NA	0.298935	601639.4
	0.142	NA	0.298935	624779.4

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